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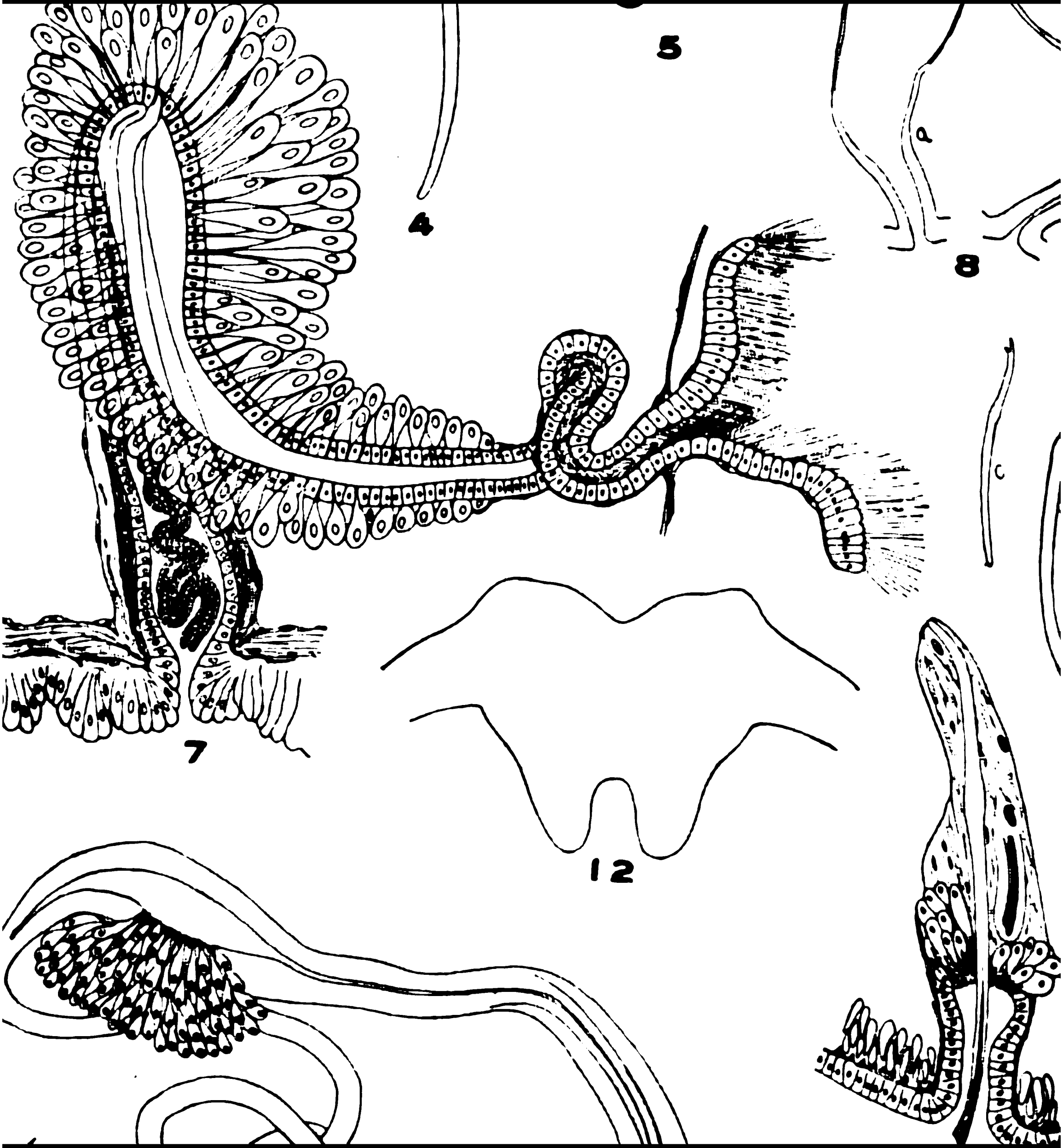

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ORGAN-FORMING SUBSTANCES IN THE EGGS OF ASCIDIANS.

EDWIN G CONKLIN.

WITH 24 PHOTOMICROGRAPHS OF LIVING EGGS OF CYNTHIA (STYELA) PARTITA STIMPSON.

BY KATHARINE FOOT AND E. C. STROBELL.

That the egg of any animal is composed of "simple, undifferentiated protoplasm" is an article of traditional belief with a large number of zoölogists, and that the cleavage of the egg is "a mere sundering of homogenous materials capable of any fate" is a doctrine which has been given great prominence in recent years. In favor of these commonly accepted views stands a considerable body of experimental work on the development of the ovum; fragments of eggs or isolated blastomeres in many cases are said to give rise to entire larvæ, thus proving, as is usually claimed, that the parts of the egg or embryo are still undifferentiated at the time of the experiment.

But not all experiments on development have confirmed these conclusions; some of the first and most careful researches of this sort led to directly opposite results. In the development of the frog's egg Roux found (1883, 85, 87, 89, 92, 93, 94, etc.) that the median plane of the embryo is determined in the egg immediately after fertilization and that "the development is, from the second cleavage on, a mosaic work of at least four vertical independently developing pieces." In 1887 Chabry showed that the early cleavage cells of the ascidian egg are specified for particular ends and that they develop, if they develop at all, into parts which they would produce under normal conditions. These results were, however, denied on the ground of other experimental work, and by many it was held that the "mosaic theory had received its death blow from the facts of experimental embryology." Among the ova in which isolated blastomeres were found to be capable of complete development were those of ascidians and from this fact it was assumed that here also the early cleavage cells were undifferentiated.

Nevertheless, although some of the results of experimental embryology seemed to render the mosaic theory untenable, additionable evidence in favor of it was furnished by another line of work commonly known as "cell-lineage." In a considerable number of animals belonging to distinct phyla (annelids, mollusks, polyclades, nemerteans, nematodes, rotifers, crustaceans and ascidians) the cleavage of the egg was found to be constant in form and differential in character and each of the early cleavage cells was shown to play a perfectly definite part in the building of the embryo. By those who maintained the traditional view as to the simplicity of the egg and the homogeneity of the blastomeres this fact was explained as due to "the continuity of development," "the position of the blastomeres in the cell complex," etc. But this explanation was never a satisfactory one and is no longer tenable; both observation and experiment have shown conclusively that in certain eggs the blastomeres are not all alike. In particular the experimental work of Crampton (1896), Fischel (1897, 1898), Boveri (1901), Wilson (1903, 1904), Yatsu (1904) and Zelney (1904) has demonstrated that in ctenophores, echinoderms, nemerteans, mollusks and annelids all portions of the egg are not equipotential; this, as well as other work on the organization of the egg, proves that there is a differentiation and localization of the substances of the egg very unlike the "simple undifferentiated protoplasm" of traditional belief.

In most instances the protoplasm of the different blastomeres of an egg is much the same in appearance; in a few cases it is visibly different, but in all cases which have been carefully studied definite blastomeres always give rise to definite parts of the embryo. In fact the manner and rate of development as well as its results are so thoroughly characteristic of certain blastomeres that students of cell-lineage have usually concluded that the protoplasm of different blastomeres must differ, even though these differences are not directly visible. Recent experimental work on some of these forms confirms and extends these conclusions and proves that even in the egg before cleavage begins different substances may be present which are destined in the course of development to enter into specific parts of the embryo.

The most notable differentiations of the oöplasm which have been observed hitherto are found in *Myzostoma* (Driesch, 1896; Wheeler, 1897; Carazzi, 1904), in *Strongylocentrotus* (Boveri, 1901), in *Unio* and *Chætopterus* (Lillie, 1901, 1902), in *Dentalium* and *Patella* (Wilson, 1904) and in the gasteropods *Crepidula*, *Physa*, *Planorbis* and *Limnæa* (Conklin, 1902, 1903). In none of these cases, however, are the differentiations and localizations of the oöplasm so remarkable as are those which occur in the ascidian egg. Here the different substances of the egg are strikingly dissimilar; they are localized in their definitive positions at a remarkably early period, and they may be traced with ease and certainty through the maturation and fertilization, the cleavage, the gastrulation and the later stages until they give rise to specific organs or parts of the larva.

Material and Methods.

I have studied the early differentiations of the egg in three species of simple ascidians, viz., *Cynthia* (*Styela*) *partita* Stimpson, *Ciona intestinalis* (L.) Flemming, *Molgula manhattensis* Verrill. The differentiations and localizations are essentially the same in all of these species, but as the different kinds of oöplasm are more brilliantly colored in *Cynthia* than in either of the other genera named, I shall devote particular attention to this form.

In an extensive publication on the organization and cell-lineage of the ascidian egg (Conklin, 1905), I have figured and described the remarkable localization of germinal materials in the egg of *Cynthia*. The differentiations of the egg substance are here so great and their localization so precise that the figures and descriptions of these might well seem to be exaggerated. I therefore welcome the opportunity of publishing a series of photomicrographs of these eggs, an opportunity which I owe to the skill and courtesy of Misses Foot and Strobell. Their method of photomicrography, which they have fully described in previous publications (Foot and Strobell, 1899, 1900, 1901, 1902) is, in some respects, the most satisfactory ever devised and yet it is as simple as it is complete. The distinguishing feature of this method consists in the accuracy and rapidity with which an exact focus can be obtained; this fact will be better appreciated when the photographs which illustrate this paper are examined in detail and when it is said that thirty such photographs, all of them satisfactory, were taken in less than four hours. With more time and material a more complete series of stages might have been photographed, but this series is sufficiently complete to show the principal features in the differentiation and localization of the egg substances.

All of the photographs are of living eggs in sea water and were taken with a Zeiss Apochromat Obj. 16 mm., Compensating Oc. 4, the bellows length being sufficient to make the magnification 112 diameters. The photographs have not been reduced in the process of reproduction and *neither the negatives nor prints were retouched or altered in any respect whatever.*

The eggs photographed were artificially fertilized and the earliest stage shown (Photo 1) was taken about three quarters of an hour after the sperm were mixed with the ova, but not more than fifteen minutes after the entrance of the spermatozoön into this particular egg. The method of procedure was to place a large drop of sea water containing a considerable number of eggs on a slide and cover with a glass supported by glass "feet" 170 μ thick (the eggs are about 150 μ in diameter). Suitable eggs were then selected and photographed by daylight, the exposure ranging from ten seconds to one minute; the shorter exposure was found to be sufficient while the longer was greatly overtimed (Photos 1 and 13). In order to obtain good color values it is necessary to have the diaphragm rather widely open and this renders the definition somewhat less distinct than it otherwise would be. Furthermore a low magnification was necessary in order to secure sufficient depth of focus to show the entire egg; even with the power employed it was not possible to bring the whole of the egg into good focus at one time. In spite of these evident disadvantages the photographs are really remarkable. Few, if any, other eggs are known in which the organization is so notable as in *Cynthia* and certainly nothing like this has ever before been photographed in the living condition. I wish here to express my great indebtedness to Misses Foot and Strobell for their kindness in making the photographs and also in superintending the production of the plate.

Differentiations and Localizations of the Oöplasm.

In the ovarian egg of *Cynthia* there is a peripheral layer of protoplasm, free from yolk, in which the "test cells" are imbedded and which contains numerous orange-yellow pigment granules which are uniformly distributed. The central portion of the egg consists of yolk-laden protoplasm which is slate-gray in color and lying somewhat eccentrically within the egg is the large, clear germinal vesicle. Before the egg leaves the ovary the chorion is formed and the "test cells" are extruded into the space between the egg and the chorion.

After the egg is laid the germinal vesicle breaks down, but the polar bodies are not formed until after the egg is fertilized. If fertilization does not take place the polar bodies are never extruded, and the clear, the yellow and the gray substances remain in the positions in which they were before the wall of the germinal vesicle disappears. If the egg is fertilized, however, a most astonishing series of movements occur which lead to the localization of the different oöplasmic substances in definite regions of the egg.

The spermatozoön always enters the egg near the vegetal pole and immediately after its entrance the peripheral layer of yellow protoplasm flows rapidly to this pole where it collects as a cap[1] (Photos 1 and 2). At the same time the clear substance derived from the germinal vesicle also flows to the lower pole where it lies between the cap of yellow protoplasm below and the yolk above. This streaming of protoplasm to the lower pole takes place so rapidly that its movements can be directly observed. Within ten or fifteen minutes after the entrance of the sperm into the egg all of the clear and yellow protoplasm has collected at the lower pole while the opposite pole where the polar bodies are formed is at this stage rich in yolk and is slate-gray in color.

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[1] In all the photographs the yellow substance appears very dark, the gray substance less dark while the clear protoplasm is relatively light.

After the clear and the yellow substances have collected at the lower pole the sperm nucleus and aster move toward one side of the egg which future development shows to be the posterior pole; the clear and yellow substances are also drawn over to this side of the egg and in such a manner that the yellow cap is transformed into a superficial band or crescent which lies just below the equator of the egg on the posterior side, its arms extending forward on each side about half way around the egg (Photos 3 and 4). Owing, perhaps, to the way in which this crescent is formed from the cap of yellow protoplasm its ventral border is sharper and its substance is of a deeper yellow than the dorsal border. At the middle of the yellow crescent is a small area of clear protoplasm which first gathers around the sperm as it enters the egg and which afterward lies at the middle of the crescent throughout the entire development; this clear protoplasm is seen in profile at the middle of the crescent in Photo 3.

The movement of the protoplasm to the posterior pole is apparently initiated by the movement of the sperm nucleus and aster to this pole; here the sperm aster divides giving rise to the amphiaster and here the two germ nuclei meet. The axis of elongation of the amphiaster is always at right angles to the axis which connects the animal and vegetal poles while its middle lies in the plane of the first cleavage and in the median plane of the embryo. The amphiaster lies beneath the yellow crescent and some distance from the surface of the egg and the long axes of the two coincide. In Photo 4 the clear line in the middle of the crescent is the amphiaster seen through the superficial layer of the crescent. It is probable that there is some causal connection between the elongation of the amphiaster and the formation of the crescent.

The clear protoplasm which also moves to the posterior pole along with the yellow is chiefly withdrawn from the surface and aggregated around the sperm nucleus and aster, though a portion of it comes to the surface just above (ventral to) the crescent (Photo 3). As the time for the first cleavage approaches the germ nuclei and amphiaster move to the center of the egg and the clear protoplasm goes with them. Finally in the telophase of the first cleavage the clear protoplasm moves into the upper (animal) hemisphere, while the yellow and gray protoplasm are situated in the lower (vegetal) hemisphere.

The first cleavage cuts through the middle of the crescent, the clear protoplasm, and the yolk, the division of all the egg substances being bilaterally symmetrical. *At the close of this cleavage each of these substances occupies its definitive position in the egg (Photos 6–8). The upper clear half of the egg gives rise to ectoderm; the crescent of yellow protoplasm surrounds the posterior side of the egg just below the equator and is later transformed into the muscle and mesenchyme cells of the larva; the gray protoplasm occupies the remainder of the lower hemisphere and gives rise to the endoderm, to the chorda, and to the neural-plate.* Two areas are distinguishable in the gray substance, though I had failed to see them until my attention was called to them by the photographs; the posterior part of the gray material lying in front of the crescent and extending some distance anterior to the vegetal pole is deeper in color and contains more yolk than the anterior portion; the latter forms a light gray crescent around the anterior border of the vegetal hemisphere, just as the yellow protoplasm forms the yellow crescent around its posterior border. The 2-cell stage when seen from the right or left sides (right side in Photo 8) shows all of these areas distinctly, the yellow crescent at the posterior pole (very dark in the photograph), the deep gray material anterior to this, and the light gray crescent occupying the anterior third of the vegetal hemisphere and extending a little above the equator, while the clear protoplasm is located chiefly in the upper hemisphere. The dark gray portion of the vegetal hemisphere gives rise to the endoderm of the larva, the light gray crescent to the notochord and neural plate.[1] *All the principal organs of the larva in their definitive positions and proportions are here marked out in the 2-cell stage by distinct kinds of protoplasm!*

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[1] It is an interesting question whether the chorda-neural-plate crescent of the ascidian egg corresponds to the "gray crescent" of the frog's egg. In both cases it lies on the anterior side of the egg and in the vicinity of the dorsal lip of the blastopore, but it is not positively known that chorda and neural plate are derived from it in the frog, as is the case in the ascidian, though this seems probable.

Although these different oöplasmic substances are chiefly localized in certain regions of the egg, which give rise to certain portions of the embryo, this segregation is not quite complete. Most of the clear protoplasm is found in the upper (ectodermal) half of the egg but some of it is also present in the lower half. Most of the yolk is found in the lower (endodermal) half of the egg, but a little of it is found in the upper half. Almost all of the yellow protoplasm is located in the mesodermal crescent, but a very small amount of it is found around the nuclei of all the cells. Thus samples of all of these egg substances are contained in all of the cells; nevertheless the segregation is so nearly complete that the clear, the gray, the light gray and the yellow areas are marked out with the greatest distinctness (Photos 7, 8).

In the 4-cell stage, as shown by Photos 9 and 10, the distribution of these substances remains as in the 2-cell stage, the yellow crescent being confined to the vegetal hemisphere and the posterior quadrants, the gray crescent to the vegetal hemisphere and the anterior quadrants, while the deep gray, yolk-laden substance lies between these crescents at the vegetal pole and the clear protoplasm occupies most of the animal hemisphere of the egg.

In the 8-cell stage the localization of these substances is the same as in the preceding stages, the clear protoplasm lying above the third cleavage plane and the other substances below it (Photo 11). The perfectly sharp boundaries of the crescent do not coincide with any of the cell boundaries, gray substance being found in the posterior dorsal cells above, below, and anterior to the crescent (Photo 11). The clear notch in the posterior profile of the crescent in Photo 11 is a cap of the same clear protoplasm which gathered around the sperm head at its entrance and afterwards lay at the middle of the crescent (Photo 3). In the 8-cell stage this clear protoplasm takes the form of two caps on the surface of the yellow crescent and adjoining, on each side, the median plane. In this same stage a small amount of yellow protoplasm may be seen around the nuclei of all the cells (Photo 11). This perinuclear yellow substance is most abundant in the posterior-ventral and in the anterior-dorsal cells; in the former it lies chiefly on the dorsal and lateral sides of the nuclei, in the latter on the posterior and lateral sides. In subsequent divisions of these cells this perinuclear plasm retains these positions and therefore goes into certain daughter cells and not into others. Consequently even in those cells in which it is found very sparingly this yellow protoplasm is localized with great definiteness.

In the 16-cell stage all these different substances remain in exactly the same positions which they held at the 2-, the 4- and the 8-cell stages (Photos 12–14). The eight ventral cells are all similar in appearance and are composed chiefly of clear protoplasm. The yellow crescent still surrounds the posterior half of the dorsal hemisphere, as in the 1-cell stage, but it is now contained in four cells; it occupies the posterior and lateral portions of these cells while the anterior and median portions are composed of gray substance. The boundary between these two substances could not be sharper if they were actually, as at first sight they seem to be, separated by a cell wall (Photo 14); only at the next stage, however, are these substances segregated into separate cells. The gray crescent occupies the anterior portions of the four anterior cells of the dorsal hemisphere, the posterior portions of these cells being composed of deep gray (endodermal) substance (Photo 14).

At the 32-cell stage (Photos 15, 16) the substances of the yellow and the gray crescents are finally segregated into separate cells, although a small portion of deep gray substance is still contained in the median cells of the yellow crescent; however this gray material moves in from the surface so that it does not show in photographs of the entire egg. The yellow crescent now consists of six cells, four median ones which are small and one pair of lateral ones which are relatively large (Photos 15, 16). The gray crescent consists of four cells of equal size and similar constitution. The ventral half of each cell is clear, contains little yolk and gives rise to most of the neural plate, the dorsal half is light gray in color, is yolk-laden and gives rise to the chorda. From this stage onward the ventral hemisphere is composed of clear cells, all of which are much alike; for this reason no photographs are given of the later stages of these cells.

In subsequent stages all of the cells of the gray and yellow crescents divide in a vertical direction (parallel to the egg axis) these divisions occur first in the gray crescent and in the most anterior pair of cells of the yellow, then in the pair of yellow cells adjoining the latter behind, and finally in the posterior median pair. The subdivision of the cells of the gray crescent occurs at the 44-cell stage; the ventral product in each case is a small clear cell which ultimately forms the posterior portion of the neural plate, the dorsal products are larger and are gray in color and ultimately develop into the chorda.

In the case of the yellow crescent both products of the posterior median cells give rise to mesenchyme, but the ventral ones contain those caps of clear protoplasm which were visible in the 8-cell stage (Photo 11) and which in a still earlier stage first appeared around the entering spermatozoön (Photo 3). The dorsal products of the other two pairs of crescent cells give rise to mesenchyme, while from the ventral halves come the muscle cells of the tadpole's tail. The mesenchyme cells are clear and faintly yellow in color, the muscle cells are a deep yellow and these two substances are distinguishable in their definitive positions even as early as the 1-cell stage (Photo 3).

Unfortunately no photographs of the early stages of gastrulation were taken. I have however studied and drawn every step of this process both in living and in prepared material. Gastrulation begins with the depression of the endoderm cells just posterior to the chorda cells, and is later continued by the rolling in of cells around the margin of the blastopore. In this manner the neural plate cells come to overlie the chorda cells, and the muscle cells, the mesenchyme.

In the closure of the blastopore the posterior (ventral) lip remains stationary until the last stages of the process, while the anterior (dorsal) lip grows backward over the gastrocoel until the blastopore is reduced to a longitudinal groove between the muscle cells of each side (Photo 17). In the overgrowth of the dorsal lip the rows of muscle cells as well as the blastopore groove are forced to the hinder end of the embryo and the muscle rows are tilted up at their anterior ends until they are transverse to the long axis (Photo 17). Later the ventral lip overgrows the remnant of the blastopore and the ectoderm of this lip forms a pair of V-shaped folds which fuse from behind forward and thus cover the dorsal lip and roll the neural plate up into a tube. In this overgrowth of the ventral lip the transverse rows of muscle cells again assume an antero-posterior direction in the embryo (Photos 18–21).

Since the neural plate is composed of relatively transparent cells which overlie the gray chorda plate, it is not well shown in the photographs, unless seen in profile (Photos 22, 23). In the dorsal view shown in Photo 18 seven or eight transverse rows of cells may be indistinctly seen in the neural plate. The chorda plate, which contains considerable yolk and is gray in color lies under the neural plate. It consists at first of a single transverse row of eight cells, then by the division of these cells two such rows are formed and finally by shoving together from the sides this plate becomes much narrower and longer. In later stages the chorda plate and the neural plate push back between the muscle cells of each side until they reach the hinder end of the embryo, thus establishing the characteristic appearance of the young larva shown in Photo 21. It can now be seen distinctly that the deep yellow cells have become the lateral muscles in the tail, that the light gray cells of the chorda plate have formed the fusiform chorda which lies between the muscle cells, and that the deep gray cells form the gastral endoderm. At the posterior end of the chorda is a group of light yellow cells which connect the muscle rows of the right and left sides; these are the caudal mesenchyme cells. At the anterior ends of the muscle rows are the clear areas of the trunk mesenchyme, which are also of a faint yellow color, while around the entire embryo is a clear layer of ectoderm cells (Photo 21).

The form of the larva is now well established and subsequent development changes this form only in minor features. In Photo 23 the tail is much elongated and bent toward the ventral side. Three rows of muscle cells with clear nuclei can be seen on the left side of the tail, while the larva is tilted toward the left so that the dorsal row of muscle cells of the right side is also visible; with the elongation of the tail the individual muscle cells have become much longer than in previous stages. Between the dorsal rows of muscle cells of the right and left sides is a clear line which is the neural tube; anteriorly this tube overlies the dark gray endoderm and hence it is not clearly visible in the photograph, but its anterior end appears as a clear, triangular area, notched where the tube is still open at the anterior end of the larva. This clear, transparent condition of the nervous system, both in the tail and trunk regions shows that the yellow protoplasm does not enter into its formation and that the muscle cells are not "neuro-muscular" cells as claimed by Castle (1896). In this and the following photograph (Photo 24) the larva is well developed, though the sense organs have not yet appeared in the sense vesicle. The most important organs of the larva are here clearly recognizable in the photographs of the living tadpoles, viz., the muscles, the notochord, the central nervous system, the gastral endoderm, the caudal and trunk mesenchyme and the ectoderm. *The substance of each of these organs is peculiar in color and constitution and these different substances may all be traced back to the 2-cell stage, where they occupy positions corresponding to their ultimate locations in the larva, while the substances of the ectoderm mesoderm and endoderm are recognizable in the unsegmented egg.* With the exception of the early gastrula stages, which were not photographed, every important step in the transformation of these substances into the organs named can be followed in the photographs of the living eggs and embryos!

Nature and Potency of the Oöplasmic Substances.

The fact that definite blastomeres of the ascidian egg give rise to definite portions of the larva has long been known (Van Beneden and Julin, 1884; Castle, 1896). Furthermore Chabry (1887) found that when certain blastomeres were killed the remaining ones gave rise only to a partial larva. On the other hand, Driesch (1895, 1903) and Crampton (1897) found that individual blastomeres of the ascidian egg developed into entire larvæ. The mere observation of the egg of *Cynthia* shows that certain areas are marked out from the time of fertilization, or even earlier, by distinct kinds of protoplasm and that these areas give rise in the course of normal development to definite organs. But, in view of the work of Driesch and Crampton, by what right are these areas called organ-forming regions and what is the justification for calling the substances of these areas organ-forming substances? The answer to both of these questions is the same, viz., in the absence of a region or substance, the organ to which it would normally give rise is not produced; and conversely each substance develops, if it develops at all, into the parts which it would normally produce. Experiments which I have carried out on ascidian eggs[1] show that the development of isolated blastomeres is strictly partial, as was first shown by Chabry and afterwards denied by Driesch. As yet I have been unable to get the isolated substances of the unsegmented egg to develop at all, but when they are isolated during the cleavage stages they develop only into the parts which they would normally produce, while the portions of the egg or embryo which lack these substances develop into embryos which lack the corresponding organs. Since the first cleavage of the egg is bilaterally symmetrical and divides all the substances of the egg equally, each of the first two blastomeres contains one half of all the organ-forming regions and substances; and since isolated blastomeres of the ascidian egg always produce rounded masses of cells which tend to close over the injured surface, many of these half embryos have the appearance of whole embryos of half size; but a careful study of living material as well as of stained preparations and sections shows that the larvæ are still incomplete up to the time of the metamorphosis. When the division of the egg or embryo is made along any other plane than the median one nothing even remotely resembling a normal larva is obtained. Every substance of the egg develops, if it develops at all, into the organs which it would normally produce, and while it has not been possible to isolate these substances in the unsegmented egg, their appearance is the same before and after cleavage begins and under these circumstances there is small room for doubting that even in the unsegmented egg these are actually organ-forming substances.

Therefore in the unsegmented egg and early cleavage stages of *Cynthia partita* we have the most complete differentiation and localization of the oöplasm ever yet reported for any egg. Apart from the nuclei, the centrosomes and the asters, there are visible in the 2-cell stage six different kinds of cytoplasmic substance,

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[1] These experiments will be published in full elsewhere.

each of which gives rise to some specific portion of the larva and is here present in its definitive position and proportions, viz., the clear protoplasm which gives rise to the ectoderm, the gray substance which produces endoderm, the deep yellow substance which develops into the muscle cells, the light yellow which goes into the mesenchyme, the clear protoplasm at the middle of the yellow crescent which becomes caudal mesenchyme and the light gray substance of the gray crescent which gives rise to chorda and neural plate. Inasmuch as it is difficult to refer to these different substances of the egg by the purely descriptive terms which have been employed thus far, I propose to designate them by names suggestive of the parts to which they ultimately give rise, viz., ectoplasm, endoplasm, myoplasm, chymoplasm, caudal chymoplasm and chorda-neuroplasm.[1] Of all of these substances the mesoplasm (myoplasm and chymoplasm) alone takes its definitive position before the first cleavage; the other substances reach their final positions only at the close of this cleavage. But although the localization is not complete in the unsegmented egg the ectoplasm and endoplasm are nevertheless clearly differentiated before cleavage begins; I am unable to say whether the chorda-neuroplasm is also differentiated at this stage.

CLEAVAGE AND GERMINAL LOCALIZATION.

In the early stages of development it is apparent that the cleavage planes do not closely follow the lines of separation between the different substances of the egg. The yellow crescent is bisected at the first cleavage; the second cleavage passes anterior to it; the third cleavage plane lies some distance above (ventral to) the upper border of the crescent; the fourth cleavage bisects the halves of the crescent on each side of the median plane. No one of these first four cleavage planes follows any one of the boundaries of the crescent; the same is also true of all the other oöplasmic substances. Although the localization of these substances is precise and definite, the localization pattern does not correspond to the early cleavage pattern. In the later cleavages some of the division walls do closely correspond with the planes

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[1] The substances of the chorda and neural plate are not clearly distinguishable from each other before the 8-cell or 16-cell stage.

of separation between these substances so that these various substances are ultimately segregated into definite cells, but this perfectly definite type of localization arises without reference to cell division and is not appreciably altered by subsequent divisions. During the first cleavage the yellow crescent substance may be seen to be undergoing complex vortical movements, but this does not permanently change the form or position of the crescent. In the unsegmented egg and in all the subsequent stages of the cleavage the yellow crescent occupies its initial position on the posterior side of the egg below the equator, irrespective of the position of the cleavage planes. Likewise the gray crescent, the ectoplasm and the endoplasm occupy the same positions in the egg at the beginning of gastrulation as in the 2-cell stage; in fact so far as localization is concerned the condition at the close of cleavage is the same as at its beginning.

CYTOPLASMIC AND NUCLEAR ORGANIZATION.

All of these different organ-forming substances are present, and are shown in the photographs, as early as the close of the first cleavage, some of them much earlier. In fact the clear ectoplasm, the gray endoplasm and the yellow mesoplasm are recognizable in the ovarian egg. Here the mesoplasm forms a peripheral layer around the whole egg in which the "test cells" are imbedded, the ectoplasm is contained within the large germinal vesicle, while the endoplasm occupies the remainder of the cell. Tracing these differentiations still further back it is found that at least a portion of the mesoplasm comes from the sphere substance (archoplasm), which is probably derived in part from the nucleus of the last oögonic division (Conklin, 1902). The yolk also is formed by the activity of the "yolk matrix" (Crampton, 1899) or yolk nucleus which is probably derived from the sphere substance. Portions of the ectoplasm, mesoplasm and endoplasm[1] are thus derived from the nucleus, the first from the nucleus of the oöcyte, the last two from the nucleus at the last oögonic division.

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[1] It may of course be objected that the yellow, the gray, and the clear substances of the ovarian egg have not been proven to be differentiated for particular ends and this I freely grant to be the case. Furthermore I do not see how this question could be tested experimentally, especially in the case of the immature ovarian egg. The fact that these substances are visibly different from one another in the oöcyte and that in all respects they resemble the ectoplasm, mesoplasm and endoplasm of the cleavage stages, to which they ultimately give rise, is the only reason for continuing to call them by these names in this earlier stage.

This remarkable condition in which considerable portions of the oöplasm can be traced back to the nucleus is of the greatest theoretical importance. From all sides the evidence has accumulated that the chromosomes are the principal seat of the inheritance material until now this theory practically amounts to a demonstration. On the other hand all persons who have much studied cell-lineage have been impressed with the fact that polarity, symmetry, differentiation and localization are first visible in the cytoplasm and that the positions and proportions of embryonic parts are dependent upon the location and size of certain blastomeres or cytoplasmic areas. However in the fact that large quantities of "nuclear sap" containing dissolved oxychromatin escape into the cell body at every mitosis (*v.* Conklin, 1902) and that these nuclear substances then contribute to the formation of specific organ-forming substances of the cytoplasm we see a possible means of harmonizing the facts of cytoplasmic organization with the nuclear inheritance theory.

Types of Germinal Organization.

By those who maintain the view that the egg is typically composed of "simple undifferentiated protoplasm" the remarkable organization of the ascidian egg will probably be regarded as an extreme case of precocious differentiation. This may perhaps be the case but the fact that germinal differentiations and localizations occur in the eggs of annelids, mollusks, nemerteans, echinoderms, ctenophores, nematodes and ascidians shows that it is by no means a rare phenomenon and it really seems as if the burden of proof were shifted to those who maintain that the egg is typically undifferentiated. Unquestionably the egg is less highly differentiated than the embryo or larva, as organ-forming substances are simpler than the organs to which they give rise, but the evidence drawn both from observation and experiment shows conclusively that in a large number of animals the substances of the egg are not homogeneous nor equipotential. But even granting that there are cases in which there is no such differentiation of the oöplasm, this supposed lack of differentiation can apply only to portions of the egg, the cytoplasm for instance, for of course there must be determinative factors ("determinants") somewhere in the ovum, probably in the nucleus which differ from one another in kind.

However in the phyla named the localization of morphogenic substances in the cytoplasm is sufficiently definite to warrant a comparison of one group with another. In all the ascidians which I have studied and apparently in all which have been studied hitherto, the type of localization is the same. Furthermore there is good reason for supposing that this type is essentially like that of *Amphioxus* and Amphibia (on this subject see Conklin, 1905). Judging from the work which has been done on the organization of the egg in other phyla, this chordate type is very distinct from that of annelids, mollusks, nemerteans, echinoderms, nematodes or ctenophores. In fact it seems necessary to recognize several distinct types of localization.

If one has regard only to the localization of the substances of the germinal layers there is considerable uniformity among most metazoa in their pregastrular stages. In almost all cases the ectodermal substances are localized in that hemisphere of the egg which is nearest the polar bodies, and in this the ascidians are no exception to the rule; in many cases the mesodermal substances are at first localized at the opposite pole, though only among the echinoderms (*Strongylocentrotus*, Boveri, 1901) is this localization persistent; among annelids, mollusks, and ascidians the mesodermal substances early move from this pole to the posterior side of the egg.

However in the localization of specific organ bases there are many notable differences among these phyla. In this regard the annelids and mollusks and perhaps the nemerteans belong to one type, the chordates, nematodes and ctenophores to entirely different types; in fact the localization of organ bases in the ascidian egg does not resemble that in the other phyla named any more closely than does the localization of the larval or adult organs of these phyla; indeed, the principal chordate features are already represented in the ascidian egg by characteristically localized organ bases as early as the 2-cell stage.

Since the time of Cuvier the principal criterion of homology has been, in the words of Owen, "correspondence in the relative position and connexion of parts." Such correspondence has been found to be much more fundamental than resemblances in size, proportions, or details of structure. Similarly in germinal organization it seems probable that the relative positions and connections of organ bases are essentially alike in different members of a phylum, though in other respects, they may vary widely. A peculiar type of localization of organ bases is thoroughly characteristic of the ascidian egg and probably the same thing is true of other phyla. If this be true, different phyla do not approach one another more closely in the earliest stages of germinal localization than in the cleavage or gastrular stages.

ORIGIN AND EVOLUTION OF GERMINAL ORGANIZATION.

The fact that there are various types of germinal localization corresponding to different types of adult organization will be explained by most persons as due to the gradual "acceleration" of development, or the shifting of adult characters back to earlier and earlier stages of the ontogeny. It is but natural that those whose attention is focused upon adult structures should regard the adult as primary, the germ as secondary; but it is surprising that embryologists also have almost universally held a similar view. Even students of cell-lineage and of the organization of the egg have generally regarded this organization as secondary and have explained it as the result of "precocious segregation," or of the "reflection of larval or adult characters back upon the egg."

Such conclusions are not founded upon observation nor experiment but upon preconceived notions as to the importance of the adult and the extreme simplicity of the germ. The whole life cycle is commonly viewed from the standpoint of the adult, and all other stages are supposed to exist *for the purpose* of leading up to a definite end stage. Similarly evolution is looked upon as the transmutation of definite end stages into others by direct modification of certain adult structures, which in some way or other modify the germ and thus become inherited.

But what is the evidence that, in either ontogeny or phylogeny, the adult is primary and the germ secondary? What is the ground for supposing that evolutionary changes first occur in the end stages and only later affect the earlier stages in the life cycle? In spite of the age-long controversy as to the inheritance or non-inheritance of acquired characters there is no satisfactory evidence that particular modifications of any adult part ever produce specific modifications of the germ. All the evidence available seems to show that the soma stands to the germ in the relation of environment and that the only influence exercised by the former upon the latter is of a general character, as Weismann has so ably argued.[1] Furthermore the difficulty of conceiving of any method by which adult characters might be transferred to the germ is well known. No hypothesis ever yet proposed for the solution of this problem harmonizes with the established facts of oögenesis and spermatogenesis. If such transfer occurs, of which there is no sufficient evidence, it can only take place by methods of which we are at present wholly ignorant.

On the other hand there is much to be said in favor of the view that the germ is primary, the adult secondary and that heritable modifications first arise in the germ and only later appear in the adult. Apart from the fact that the germ gives rise to the adult and to other germs, it is known that in certain cases apparently slight modifications of the germ may produce profound modifications of the adult, whereas the reverse is not known to be true. One of the most convincing evidences of the truth of this view is found in cases of cross breeding, particularly in hybridization, where it is certain that hybrid characters of an offspring are directly due to the hybrid character of the germ, since they can have no other possible cause. The evidence drawn from experiments on eggs and embryos on first thought seems to be conflicting; in some cases fragments of eggs or embryos give rise only to partial larvæ and injured eggs produce only embryos showing more or less serious defects; in other cases entire embryos are produced under these conditions, but these results cannot be regarded as destructive of this argument for, as has long been maintained by Roux, such cases of entire

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[1] Undoubtedly one important cause of germinal variation is to be found in the influence of changing environment upon the germ, but this is far from saying that particular modifications of the adult are transmitted to the germ.

development of egg fragments may be the result of regenerative, or regulative, processes. It is not usually possible to connect definite modifications of the adult with definite alterations of the germ from which it developed, but one remarkable instance in which this is possible is found in cases of inverse symmetry. In sinistral gasteropods, and presumably in all other cases of inverse symmetry, the cause of inversion is to be found in the inverse organization of the unsegmented egg and I have elsewhere (1903) shown reason for believing that this may be due to the maturation of the egg at opposite poles in dextral and sinistral forms. Here one of the most sudden and profound alterations of structure with which we are acquainted may be traced back to a specific modification of the germ.

These facts point to the conclusion that the complex organization of an egg, such as that of an ascidian, has not arisen through the "reflection of adult characters upon the egg," but rather that this organization is primary. Furthermore they seem to indicate that evolution has taken place, not through modifications of adult structure, but through changes in germinal organization; modifications of this organization, however produced, are probably the real causes of evolution.

This conclusion, which has grown out of a study of the complex organization of the germ and its relation to adult organization, harmonizes entirely with the mutation theory of DeVries; it indicates how mutations in elementary germinal characters may appear as widespread modifications in the mature organism; it offers an explanation of otherwise inexplicable variations of adult structure, such as inverse symmetry; and finally it suggests a possible solution of that vexed problem of the origin of phyla, not by the transmutation of one adult form into another, as is assumed in all previous hypotheses, but by relatively simple alterations of the type of germinal organization.

UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA,
December 30, 1904.

LITERATURE CITED.

Beneden, E. Van, et Julin, Ch.

'84 La segmentation chez les ascidiens dans ses rapportes avec l'organization de la larve. Arch. de Biol., V.

Boveri, Th.

'01 Ueber die Polarität Seeigeleis. Verh. Phys. Med. Ges. Würzburg, XXXIV.

'01 Die Polarität von Ovocyte, Ei, und Larve des Strongylocentrotus lividus. Zool. Jahrb., XIV.

Carazzi, D.

'04 Ricerche embriologiche e citologiche sull'uovo di Myzostoma glabrum Leuckart. Monitoro Zool. Italiano, XV.

Chabry, L.

'87 Contribution a l'embryologie normal et teratologique des ascidies simples. Jour. Anat. et Physiol., XXIII.

Castle, W. E.

'96 The Early Embryology of Ciona intestinalis Flemming (L.). Bull. Mus. Comp. Zool., XXVII.

Conklin, E. G.

'02 Karyokinesis and Cytokinesis in the Maturation, Fertilization and Cleavage of Crepidula and other Gasteropoda. Jour. Acad. Nat. Sci. Philad., XII.

'03 The Earliest Differentiations of the Egg. Abstract of Paper before American Philosophical Society. Science, XVII., No. 435.

'03 The Cause of Inverse Symmetry. Anat. Anz., XXIII.

'05 Organization and Cell-Lineage of the Ascidian Egg. Jour. Acad. Nat. Sci. Philad., XIII.

Crampton, H. E.

'96 Experimental Studies on Gasteropod Development. Arch. Entw. Mech., III.

'97 The Ascidian Half-Embryo. Ann. New York Acad. Sci., VIII.

'99 Studies upon the Early History of the Ascidian Egg. Jour. Morph., XV., Supplement.

Driesch, H.

'95 Von der Entwicklung einzelner Ascidienblastomeren. Arch. Entw. Mech., IV.

'96 Betrachtungen über die Organization des Eies und ihre Genese. Idem, IV.

'03 Ueber Anderung der Regulationsfähigkeiten im Verlauf der Entwicklung bei Ascidien. Idem, XVII.

Fischel, A.

97, '98 Experimentelle Untersuchungen am Ctenophorenei. I. and II., Arch. Entw. Mech., VI., VII.

'03 Entwicklung und Organ-Differenzirung. Idem, XV.

Foot, K., and Strobell, E. C.

'99 Further Notes on the Egg of Allolobophora fœtida. Zool. Bull., II.

'00, '01 Photographs of the Egg of Allolobophora fœtida. I. and II., Jour. Morph., XVI.

'02 A new Method of Focusing in Photomicrography. Zeit. wiss. Mikroskopie, XVIII.

Lillie, F. R.

'01 The Organization of the Egg of Unio, etc. Jour. Morph., XVII.

'02 Differentiation without Cleavage in the Egg of the Annelid Chætopterus pergamentaceus. Arch. Entw. Mech., XIV.

Roux, W.

'83 Ueber die Zeit der Bestimmung der Hauptrichtungen des Froschembryo. Gesemmelte Werke, II.

'85 Ueber die Bestimmung der Hauptrichtungen des Froschembryo im Ei und über die erste Theilung des Froscheis. Idem, II.

'87 Der Bestimmung der Medianebene des Froschembryo durch die Copulationsrichtung des Eikerns und des Spermkerns. Idem.

'92 Ueber das entwicklungsmechanische Vermögen jeder der beiden ersten Furchungszellen des Eies. Verh. d. Anat. Ges. zu Wien, 1892.

'95 Einleitung. Arch. Entw. Mech., I.

'03 Ueber die Ursachen der Bestimmung der Hauptrichtungen des Embryo im Froschei. Anat. Anz., XXIII.

Wheeler, W. M.

'97 The Maturation, Fecundation and early Cleavage in Myzostoma. Arch. de Biol., XV.

Wilson, E. B.

'03 Experiments on Cleavage and Localization in the Nemertine Egg. Arch. Entw. Mech., XVI.

'04 Experimental Studies on Germinal Localization. I. and II., Jour. Exp. Zool., I.

'04 Mosaic Development in the Annelid Egg. Science, XX., No. 518.

Yatsu, N.

'04 Experiments on the Development of Egg Fragments in Cerebratulus. Biol. Bull., VI.

Zelney, C.

'04 Experiments on the Localization of Developmental Factors in the Nemertine Egg. Jour. Exp. Zool., I.

DESCRIPTION OF PHOTOGRAPHS. (PLATE XI.)

All the photographs are of the living eggs and embryos of *Cynthia* (*Styela*) *partita* in sea water and are magnified 112 diameters. Prints from the original negatives were arranged in order and photographed and the plate is an actual print (Rotograph Process) from this negative. These photographs are not diagrams but like the specimens themselves they require and will repay careful study. That portion of the egg which appears darkest in the photographs is the orange-yellow mesoplasm, that which is lightest is the transparent ectoplasm, while that which is apparently intermediate in shade between these two is the slate-gray endoplasm. The contrast between these substances is therefore greater in reality than appears in the photographs. Every egg or embryo is inclosed in a transparent chorion which does not show in the photographs; within the chorion and around the periphery of the egg are numerous "test cells" which contain yellow pigment.

PHOTO 1. Egg about fifteen minutes after the entrance of the sperm showing the yellow protoplasm as a dark cap at the lower pole where the sperm lies. The clear protoplasm is the light zone above the yellow; the rest of the egg is gray.

PHOTO 2. Egg about twenty minutes after the entrance of the sperm; the egg substances are shown as in the preceding photograph.

PHOTO 3. Egg about thirty minutes after the entrance of the sperm, seen from the right side. The yellow protoplasm is moving to the posterior pole and forming the crescent there; the clear protoplasm lies chiefly above (ventral to) the crescent; in the middle of the crescent and at the periphery of the egg is a small spot of clear protoplasm (caudal chymoplasm) which first appears around the entering sperm and ultimately goes into a pair of caudal mesenchyme cells.

PHOTO 4. Egg about thirty-five minutes after the entrance of the sperm, showing the yellow crescent as a dark band with a clear area through its center; the latter is the first cleavage spindle. Both the crescent and the periphery of the egg show a slight notch in the lower (ventral) border, which is the beginning of the first cleavage furrow. The egg is somewhat obscured by overlying test-cells which here and elsewhere give it a mottled appearance; high focus.

PHOTO 5. Egg about forty minutes after the entrance of the sperm, viewed from the posterior-ventral pole. The cleavage furrow is deepest in the region of the crescent. Above the crescent is the clear (ventral) ectoplasm. High focus.

PHOTO 6. Egg about forty-five minutes after the entrance of the sperm. Two-cell stage viewed from the animal pole showing the yellow crescent at the posterior margin of the egg.

PHOTO 7. Stage similar to the preceding, viewed from the posterior pole; below the crescent is shown the gray endoplasm of the vetegal pole, above the crescent the clear ectoplasm of the animal hemisphere, in the furrow at the middle of the crescent a small amount of clear chymoplasm.

PHOTO 8. Stage similar to the preceding, viewed from the right side (an end view of one of two cells), showing the clear ectoplasm in the upper (animal) hemisphere, the yellow crescent (mesoplasm) at the posterior pole, the light gray crescent (chorda-neuroplasm) at the anterior pole and the dark gray endoplasm between the two crescents at the lower pole. The definitive localization of these substance is complete at this stage.

PHOTO 9. Four-cell stage from vegetal pole, showing the yellow crescent across the two posterior cells. The anterior cells lie at a lower level than the posterior ones and the focus is such that only the ends of the crescent show clearly.

PHOTO 10. Four-cell stage from the animal pole, high focus; many test cells cover the egg; the yellow crescent, which lies on the lower side of the posterior cells, shows indistinctly through the egg; an area of clear protoplasm is shown in each of the cells.

PHOTO 11. Eight-cell stage from the right side; the upper cells contain the clear ectoplasm, though a small amount of yolk is found at the periphery of each cell; most of the yellow protoplasm is contained in the yellow crescent, the outline of which is very distinct, but a small amount of yellow protoplasm is found around the nuclei of all the cells; in the posterior ventral (upper) cells this lies on the lateral and dorsal side of the nucleus, in the anterior dorsal (lower) cells it lies on the lateral and posterior side of the nucleus. At the middle of the yellow crescent and seen as a notch in its posterior outline is a small cap of caudal chymoplasm (the same as that seen in Photo 3). The yellow crescent is bounded by dark gray endoplasm which extends forward to the middle of the anterior-dorsal cells; the gray crescent of chorda-neuroplasm occupies the anterior portions of these cells. The forward slant of the vertical (second cleavage) furrow and the "cross furrow" formed by it and the third cleavage are clearly shown. Photos 3, 8, and 11 are all viewed from the right side and the localizations of the same organ-forming substances in the 1-, 2- and 8-cell stages are clearly shown in these photos.

PHOTO 12. Sixteen-cell stage from dorsal side, view slightly oblique. The eight dorsal cells are clearly shown, while three transparent ventral cells are indistinctly shown on the left-anterior periphery. The yellow crescent is contained in the four posterior cells, the lighter margins of the four anterior cells represent the gray crescent. The median cells behind are nearly filled with yellow mesoplasm, save for the clear nuclei and a small wedge of gray substance in the anterior portion of these cells; the lateral portions of the cells just anterior to these are composed of yellow substance, their median portions of gray material.

PHOTO 13. Sixteen-cell stage from the posterior pole, showing the four yellow crescent cells with clear nuclei; below them is the gray endoplasm, above them the clear ectoplasm; the ectoderm cells are indistinctly shown with a trace of yellow substance around nuclei of the four posterior cells.

PHOTO 14. Sixteen-cell stage transitional to 32-cell stage, dorsal view showing eight cells. The localization of the different oöplasmic substances is the same as in Photo 12, but the focus is a little deeper. The yellow and gray crescents are remarkably distinct; between the two is the area of deep gray endoplasm; the light area on the inner border of the yellow crescent is chymoplasm.

PHOTO 15. Thirty-two cell stage, dorsal view. The small posterior crescent cells have divided transversely, forming four small cells; the large mixed cell anterior to these is just cutting off its outer yellow portion from its inner gray one. The four anterior cells have divided in an antero-posterior direction thus separating the gray crescent of chorda-neuroplasm from the endoplasm. The nuclei in all the cells appear as clear areas.

PHOTO 16. Thirty-two cell stage, dorsal view, similar to the preceding.

PHOTO 17. Advanced gastrula, posterior view, superficial focus. The blastopore groove is a narrow slit bounded on each side by four large muscle cells, which are derived from the yellow crescent; the lighter colored cells at the bottom of the groove are mesenchyme and are derived from the middle part of the crescent. The dorsal lip of the blastopore (not clearly shown in the photo) closes the groove dorsally and anteriorly.

PHOTO 18. Late gastrula, dorsal view, superficial focus. The U-shaped group of mesoderm cells (Photo 17) is seen from the open end of the U; the ectoderm overgrowing the mesoderm is seen as a light area posterior to the yellow cells; the light wedge-shaped area in the mid-line is the beginning of the neural groove; the apex of the wedge lies between the limbs of the U and marks the point at which the blastopore closes and also the posterior limit of the neural plate. The ectoderm can be seen as a zone of clear cells with transparent nuclei around the periphery and faint indications of these cells with their clear nuclei can be seen forming seven or eight transverse rows of cells across the embryo anterior to the blastopore (the neural plate). The gray endoderm seen through these ectoderm cells gives a dark appearance to all the embryo save the periphery.

PHOTO 19. Late gastrula, dorsal view, deep focus. This stage is later than the preceding and the embryo is tilted slightly toward the right side, so that the plane of symmetry is a little to the right of the middle of the photo; the mesoderm cells are no longer transverse to the long axis but are extending in an antero-posterior direction. In front of the mesoderm is a dark area, the endoderm, in which four transverse rows of cells may be indistinctly seen; around the entire periphery is the clear ectoderm. The anterior portion of the embryo is wider and the posterior part narrower than at any previous stage.

PHOTO 20. Young tadpole, ventral view, superficial focus; the larva is slightly tilted to the right so that the ventral mid-line lies to the right of the middle of the photo. Three rows of rounded muscle cells (six or seven cells in a row), with clear nuclei, lie on each side of the mid line. In front of the muscle cells on each side is a clear area of mesenchyme. The strand of caudal endoderm cells shows in the mid-line between the muscle rows of each side; anterior to the muscle and mesenchyme is the gastral endodern.

PHOTO 21. Young tadpole, ventral view, deep focus. Between the muscle cells is the fusiform notocord which is composed of wedge-shaped cells. Six muscle cells are visible on each side of the chorda and there are several lighter colored mesenchyme cells at its posterior end. At the anterior ends of the muscle rows is a clear area of mesenchyme cells in which the peribranchial pouches appear. Cells of the gastral endoderm are clearly visible; around the entire periphery are clear ectoderm cells.

PHOTO 22. Young tadpole from left side. The three rows of muscle cells, each with a clear nucleus are faintly shown in the dark area in the tail, the gastral endoderm also appears as a dark area in the trunk, but not so dark as the muscle cells. Around the entire periphery is the clear ectoderm; on the dorsal (convex) side this is especially thick and comprises the neural tube. The hazy areas ventral to the embryo are due to aggregated test cells.

PHOTO 23. Tadpole of about the same stage as the preceding, left-dorsal aspect.

The rows of muscle cells show clearly along the left side ; the dorsalmost row of the right side also shows and between these two is a clear line, the nerve tube (or cord); anteriorly this cord leads to the wedge-shaped light area at the anterior border of the larva, which is the sense vesicle (brain) and which is still open to the exterior. The test cells somewhat obscure the posterior portion of the tail.

PHOTO 24. Tadpole a little older than the preceding viewed from the right side. The tail is much elongated and the muscle cells, each faintly marked by a clear nucleus and dark outline, are also elongated as compared with preceding photos. Two rows of muscle cells are distinctly visible, the third also comes into view at the hinder end of the tail. There are seven or eight cells in each row as indicated by the number of nuclei. The gastral endoderm shows as a dark area in the trunk. The neural cord and tube is the clear area on the dorsal side of the trunk, its anterior limit being marked by a flattened contour line where the neural folds are just closing.

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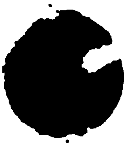
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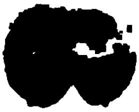
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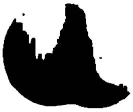
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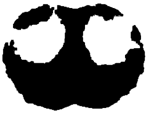
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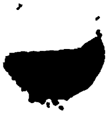
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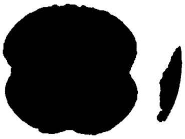
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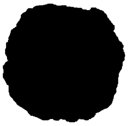
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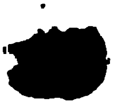
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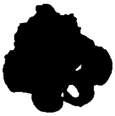
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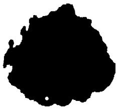
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MOSAIC DEVELOPMENT IN ASCIDIAN EGGS.

BY

EDWIN G. CONKLIN.

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In almost every instance in which fragments of eggs or isolated blastomeres have been found to be capable of giving rise to entire larvæ the substance of the unsegmented egg is apparently undifferentiated and the cleavage cells are so nearly equal and homogeneous that it has not been possible to trace the lineage of individual blastomeres throughout the development. The most notable exception to this rule is found in the case of ascidians. That the cleavage of the egg in these animals is constant in form and differential in character and that specific blastomeres are destined in the course of normal development to give rise to specific parts of the larva has been demonstrated by Van Beneden and Julin, Chabry, Castle, and many others. Chabry ('87) also showed, in one of the earliest experimental investigations dealing with the potency of cleavage cells, that individual blastomeres of Ascidia aspersa always develop into those parts of the larva which they would produce under normal conditions. On the other hand, Driesch ('95) discovered, some eight years later, that in Phallusia mammilata individual blastomeres up to the 4-cell stage at least are capable of giving rise to entire larvæ and this conclusion was afterward confirmed by Crampton ('97) in the case of Molgula manhattensis. Since the results of Chabry were thus flatly contradicted by these later investigators and as they have been defended by no one who has actually experimented on these eggs[1] these results have been generally discredited and the ascidians are now commonly regarded as belonging to that group of animals in which the early cleavage cells are equipotential. The ascidians, therefore, should afford an excellent opportunity of determining the exact method by which an egg fragment or isolated blastomere gives rise to an entire larva, since in this case it is possible to follow the lineage of individual cells until they enter into larval organs; furthermore, they should afford means of testing the justice of the distinction which has been proposed (Conklin, '97) between determinate and indeterminate types of cleavage, and finally they should throw light upon the significance of the high degree of differentiation which is known to exist in the early development of these animals.

I. NORMAL DEVELOPMENT.

I have recently ('05[1]) shown that these differentiations of the ascidian egg are much greater than has heretofore been supposed; in the unsegmented egg of Cynthia (Styela) partita at least five distinct kinds of oöplasm can be recognized. These are, (1) the deep yellow protoplasm which later enters into the muscle cells of the tail of the larva; (2) the light yellow material which becomes mesenchyme; (3) the light gray material which forms the chorda and neural plate; (4) the slate gray substance which becomes endoderm, and (5) the clear transparent protoplasm which gives rise to the general ectoderm. All of these substances are recognizable in the egg before the first cleavage and immediately after that cleavage they all occupy their definitive positions in the egg, the yellow protoplasm forming a yellow crescent around the posterior side of the egg just dorsal to the equator, the light gray substance forming a gray crescent around the anterior border of the egg, the slate gray substance lying at the middle of the dorsal hemisphere and between the two crescents, while the transparent protoplasm is chiefly localized in the ventral hemisphere of the egg. In these positions and from these substances the organs and germinal layers specified arise.

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[1] Several persons, viz: O. Hertwig ('92), Roux ('92), Weismann ('92), Barfurth ('93) have discussed Chabry's work from a critical point of view.

At the first cleavage of the egg all of these substances and areas are equally divided, since this cleavage lies in the plane of bilateral symmetry of the egg and future embryo. The second cleavage plane is perpendicular to the first and separates the gray crescent in front from the yellow crescent behind; the cells of the anterior quadrants are therefore very unlike the posterior ones and the two can always be distinguished at a glance. (Fig. 1.) The third cleavage is equatorial and separates four clear ventral cells from four dorsal ones which contain the yellow and gray crescents and the deep gray material. (Fig. 2.) The ectoplasm is now completely segregated in the four ventral cells but the other oöplasmic substances are not as yet located in separate cells, though from the time of the first cleavage onward their locations and boundaries are perfectly sharp and distinct.

At the fourth cleavage each of the eight cells divides, thus giving rise to sixteen cells (Fig. 3) and at the fifth cleavage these are increased to thirty-two. During the fifth cleavage the substance of the gray crescent is segregated into four cells ($A^{6.2}$, $A^{6.4}$, Fig. 4)[1] at the anterior border of the egg, while the yellow crescent comes

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[1] The system of cell nomenclature employed in this paper is similar to that used by Castle ('96) and is fully explained in my work on the cell-lineage ('05^1); in brief *A* and *a* designate cells of the anterior half of the egg, *B* and *b* those of the posterior half, the capitals being used for cells of the vegetal (dorsal) hemisphere, the lower case for those of the animal (ventral) hemisphere. Corresponding cells of the right and left sides receive the same designation, except that those of the right side are underscored.

Normal Development of Cynthia partita, 4-Cell to 64-Cell Stages; × 333.

The yellow crescent which surrounds the posterior half of the egg dorsal to the equator is stippled. The gray crescent around the anterior border of the egg is left unshaded. The boundary between the clear protoplasm and the yolk is indicated by a crenated line. The polar bodies (shaded by vertical lines) lie at the animal or ectodermal pole.

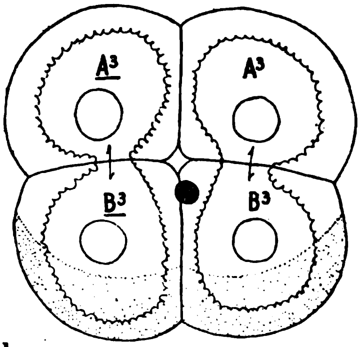
Fig. 1. Four-cell stage from the animal pole, the yellow crescent showing through the egg.

Fig. 2. Telophase of the third cleavage (8-cell stage), from the left side.

Fig. 3. Twenty-cell stage from the animal (ventral) pole.

Fig. 4. Twenty cells, transitional to the 24-cell stage, from the vegetal (dorsal) pole. The gray crescent is now segregated in the two pairs of cells $A^{6.2}$, $A^{6.4}$; the yellow crescent will be localized in separate cells at the close of the division which has already begun in the cells $B^{5.1}$.

Figs. 5 and 6. Ventral and dorsal views of the same egg in the 64-cell stage. The yellow and the gray crescents each consist of a double arc of cells; the anterior arc of the gray crescent ($A^{7.4}$, $A^{7.8}$) is composed of neural plate cells, the posterior arc ($A^{7.3}$, $A^{7.7}$), of chorda cells; only two pairs of cells in the yellow crescent ($B^{7.4}$, $B^{7.8}$) are muscle cells, the others are mesenchyme. The pair of cells $A^{7.6}$ also gives rise to mesenchyme. All the other cells of the dorsal hemisphere (Fig. 6) are endodermal. All the cells shown in Fig 5, except those of the yellow and gray crescents, are ectodermal.


A^3
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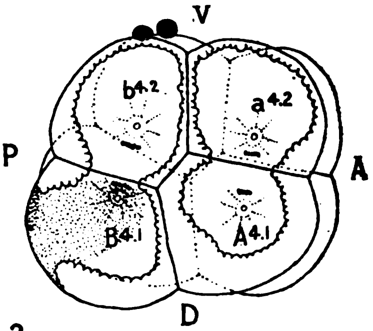
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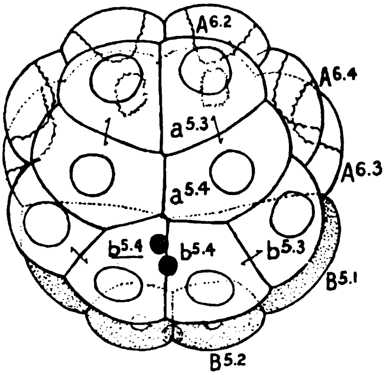
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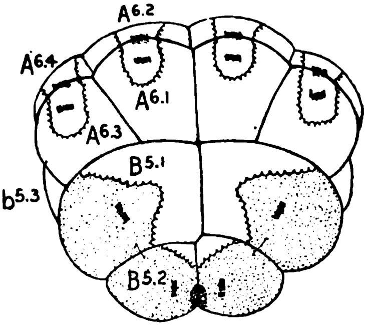
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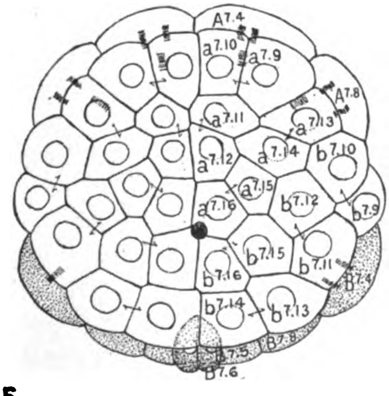
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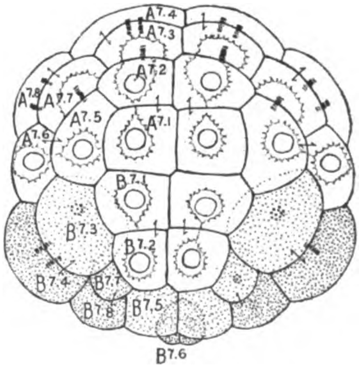
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6

to occupy six cells ($B^{6.3}$, $B^{6.4}$, $B^{6.2}$) around the posterior border (the spindles which lead to the formation of these six cells are indicated in Fig. 4). These thirty-two cells are increased to sixty-four at the next cleavage (Figs. 5 and 6); during this cleavage four chorda cells ($A^{7.3}$, $A^{7.7}$) are separated from the four neural plate cells ($A^{7.4}$, $A^{7.8}$, Fig. 6), while the six cells of the yellow crescent have given rise to twelve, four of which are muscle cells ($B^{7.4}$, $B^{7.8}$) and eight mesenchyme ($B^{7.3}$, $B^{7.7}$, $B^{7.5}$, $B^{7.6}$). At the same time an additional pair of mesenchyme cells ($A^{7.6}$) is separated from a pair of endoderm cells in the anterior quadrants. This is the only mesenchyme cell derived from the anterior quadrants.

At this stage all the substances of the germ layers and of the principal organs of the larva are gathered into separate cells, but although this segregation into separate cells comes relatively late in the cleavage these substances have been definitely localized in certain regions of the egg from the time of the first cleavage. Subsequent cleavages lead to changes in the shape of the embryo but produce no changes in this localization.

In the gastrulation the endoderm cells are depressed and are overgrown in front by the chorda cells and these in turn are covered by the neural plate cells; similarly the mesenchyme cells overgrow the endoderm at the posterior border of the blastopore, while the mesenchyme cells are overgrown by the muscle cells, and finally the latter by the ectoderm. (Figs. 7–10.) In the closure of the blastopore the anterior (dorsal) lip grows posteriorly until it covers most of the dorsal face, while the muscle cells form the lateral boundaries of the blastopore. (Figs. 9, 10.) In this overgrowth of the dorsal lip the chorda cells which originally lay at the anterior border of the egg are carried back into the posterior half of the embryo, where by interdigitation they form the chorda. The neural plate cells are also carried back with the chorda nearly to the posterior end of the embryo. The ventral (posterior) lip of the blastopore then grows forward over the remnant of the blastopore and the neural plate is rolled up into a tube which closes from behind forward. The muscle cells become arranged in three rows on each side of the chorda; in front of the muscle cells is a mass of small mesenchyme cells, while a double row of endoderm cells ventral to the chorda constitutes the cord of ventral or caudal endoderm. (Figs. 11 and 12.) Finally the tail of the larva elongates greatly and becomes coiled around the body of the larva within the egg membranes, and about twelve hours after the fertilization of the egg the larva may hatch and become free swimming. However, in a considerable proportion of cases the larva never hatches but undergoes its metamorphosis within the egg membranes.

II. OBJECTS AND METHODS OF EXPERIMENT.

This brief review of the normal development[1] shows that there is a remarkable degree of differentiation and localization of the substances of the egg and embryo and it seems to render necessary some further explanation of the results of the experiments of Driesch and Crampton; certain it is that the egg is highly differentiated and if portions of this differentiated oöplasm may give rise to portions of the larva which they would never produce under normal conditions it is important to know the steps by which this is accomplished.

With this object in view I spent the summer of 1904 at the Marine Biological Laboratory at Woods Hole, Mass., experimenting on the eggs of Cynthia (Styela) partita and of Molgula manhattensis; I was unable to obtain Ciona intestinalis, the normal development of which I had studied during the previous summer, and my experimental work is therefore limited to the two species first named. Most of my work was done on the egg of Cynthia, which is a better object for experimental work than that of Molgula, owing to its greater size and the more brilliant coloring of its different oöplasmic substances. Enough work was done on Molgula, however, to show that the development of isolated blastomeres is the same in this genus as in Cynthia.

All the experiments performed had for their purpose the testing of the potencies of the various substances and blastomeres of the egg. Injuries to the unsegmented egg of whatever nature, whether produced by sticking, cutting or shaking the eggs, invariably inhibited all further development. I have therefore been unable to test the developmental potencies of the different kinds of oöplasm of the unsegmented egg. But inasmuch as these substances are the same in appearance and localization before and

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[1] For a more detailed account of the normal development of these ascidians the reader is referred to my previous papers on the "Organization and Cell-Lineage of the Ascidian Egg" ('05[1]), and on "Organ-Forming Substances in the Eggs of Ascidians" ('05[2]).

NORMAL DEVELOPMENT OF CYNTHIA PARTITA, GASTRULA TO TADPOLE; × 333.

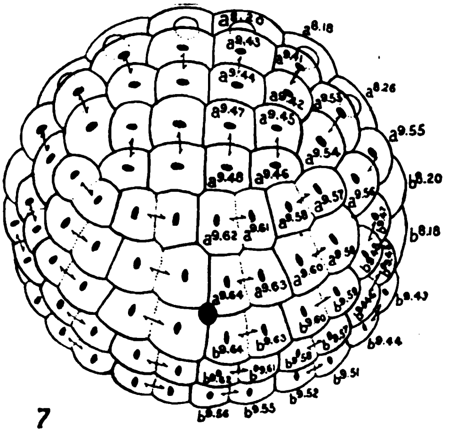
The neural plate or tube is finely stippled, the chorda coarsely stippled; muscle cells are shaded by vertical lines, mesenchyme by transverse lines.

Figs. 7 and 8. Ventral and dorsal views of a gastrula (180-cell stage), showing T-shaped blastopore, neural and chorda plates, mesenchyme and muscle cells. Most of the cleavage cells are in the ninth generation.

Figs. 9 and 10. Two views of the same gastrula from the dorsal pole; Fig. 9, showing the superficial cells, Fig. 10, those at a deeper level. The overgrowth of the dorsal lip of the blastopore and the approximation of the muscle cells of each side toward the median plane have reduced the blastopore to a longitudinal groove in the posterior half of the embryo. The ectoderm cells are in the tenth generation and there are in the entire embryo about 360 cells.

Fig. 11. Dorsal view of an embryo in which the neural plate (n. p.) is closing to form the neural tube (n. t.) Beneath the nerve tube is the notochord and on each side of the latter is shown a row of muscle cells (ms.) At the posterior end of the muscle rows is the caudal mesenchyme, at their anterior end the trunk mesenchyme (m'ch.)

Fig. 12. Young tadpole viewed from the left side, showing three rows of large muscle cells (ms.) along the side of the notochord (ch.); dorsal to the latter is the nerve tube (n. t.); anterior to the muscle rows is the trunk mesenchyme (m'ch.); ventral to them is the ventral or caudal endodem (v. end.)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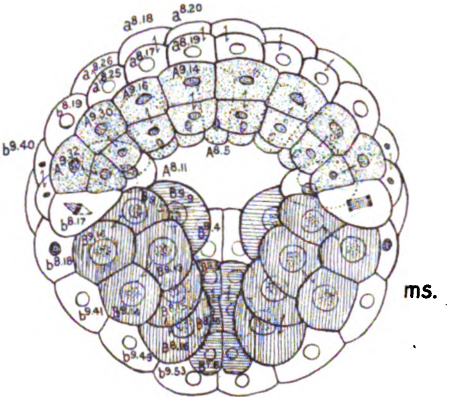
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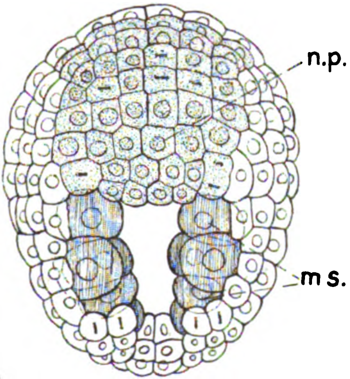
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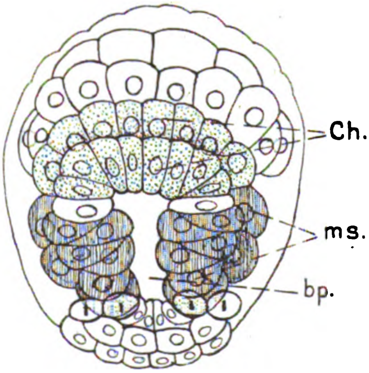
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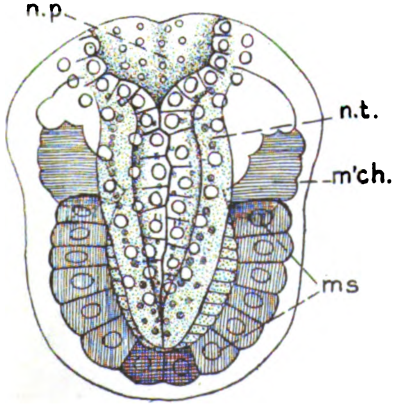
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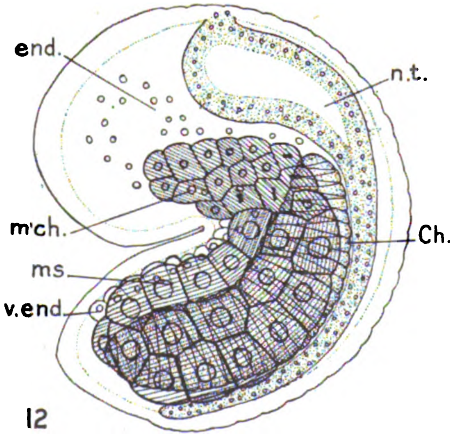
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12

after cleavage begins it can scarcely be doubted that their potencies are also the same. Hundreds of experiments involving many thousands of eggs were made upon the various cleavage stages. The methods of experimenting which I employed were essentially like those used by Driesch and Crampton, viz: the eggs in the 2-cell, 4-cell, 8-cell or later stages were strongly spurted with a pipette, or were shaken in a vial, and thereby some of the blastomeres were frequently injured while others were uninjured and continued to develop. The injured blastomeres were rarely killed, as was shown by the fact that they remained transparent and entire for a day or more, whereas dead cells soon become opaque and disintegrate. These injured cells never again divide and sections show that their nuclei are frequently broken and their chromosomes scattered. Cells are more likely to be injured during nuclear division than during rest. The fact that these injured cells never again divide though they remain whole within the chorion and preserve their characteristic color and structure makes it possible to determine at all stages just what cell or cells have been injured. Whether or not the presence of these injured cells within the chorion may influence the development of the uninjured cells will be considered later. Attempts to completely separate individual blastomeres by the use of Herbst's calcium-free sea water were not successful, probably owing to the presence of the chorion and to the close union between the blastomeres.

In addition to this method of experimentation which yielded hundreds and thousands of eggs in which one or more of the blastomeres had been injured I also cut eggs and embryos in two with knives made from small needles. In no single instance was I able to get fragments of unsegmented eggs to develop; in the gastrula stages I was more successful, being able to cut gastrulæ in two in the manner described by Driesch ('03) and observe the subsequent development.

I have not attempted to repeat the various ingenious methods of injuring blastomeres which were devised and employed by Chabry, since they are necessarily slow and difficult of application and yield but a small number of injured eggs, whereas by simply spurting or shaking the eggs one may injure blastomeres in an enormous number of eggs which can then be sorted out and classified according to the character of the injury; furthermore the ease and certainty with which the identity of injured blastomeres of

Cynthia may always be determined renders unnecessary such experiments as Chabry's on the individual cleavage cells.

If one desires to trace with accuracy the lineage of individual blastomeres, whether in normal or experimentally altered development, it is essential that a large quantity of material should be available. In even the most favorable material the lineage of the later stages can be successfully studied only by the aid of fixed and stained material and without a large number of eggs it is difficult if not impossible to secure all the stages of development. Furthermore it is desirable that a considerable number of eggs of every stage be available for study, since the liability to error decreases with the number of cases studied. Accordingly, in addition to the study of living eggs during successive stages after their injury, many eggs were also fixed at brief intervals and were afterward stained and mounted entire or sectioned. For this purpose I have found Kleinenberg's picro-sulphuric acid followed by my picro-hæmatoxylin to give the best results. Entire eggs so prepared show cell outlines, nuclei and karyokinetic figures much more plainly than in the living condition; on the other hand the yellow crescent is less distinct since the yellow pigment is extracted by alcohol; nevertheless this crescent may always be recognized by its peculiar staining qualities and it therefore affords a never failing aid in orientation.

III. RESULTS OF EXPERIMENTS.

In undertaking this work it seemed to me scarcely possible that all of these strikingly different kinds of oöplasm, each with its own peculiar developmental history and destiny, were nevertheless morphogenetically alike, as might be concluded from the results of Driesch and Crampton. On the other hand a possible escape from this conclusion was suggested by the fact that although the cleavage cells are strikingly different from one another, the isolation of the oöplasmic substances in them is not quite complete; almost all of the yellow protoplasm is contained in the yellow crescent; but a small amount of it is found around the nuclei of all the cells; most of the gray substance is contained within the dorsal hemisphere, but a small amount of it occurs in the ventral cells also; most of the clear protoplasm is found in the ventral hemisphere but a small quantity is also found in the dorsal cells.

It therefore seemed possible that the production of a complete larva from any one or two of the first four cells might be due to the replacing of a missing substance by the greater development of the trace of that substance contained in the cells in question. Thus the anterior quadrants which lack the yellow crescent might, perhaps, regenerate it from the small amount of yellow perinuclear protoplasm which they contain, and correspondingly the posterior quadrants might regenerate the lacking gray crescent from the small amount of gray substance which they contain. In the light of the work of Driesch and Crampton either there must be such regeneration, or the substances which appear so different must after all be each and all totipotent.

However the solution of this problem has turned out to be much simpler than I had supposed possible, viz: *isolated blastomeres do not give rise to entire larvæ, as claimed by Driesch and Crampton, but on the contrary each blastomere produces only those parts of a larva which would arise from it under normal conditions. The development is, in short, a "mosaic work."* Since the first cleavage is bilaterally symmetrical each of the first two blastomeres contains one-half of each and all of the substances of the egg and correspondingly the half larva which develops from one of these blastomeres contains portions of every larval organ. Owing to the fact that the cells which arise from an isolated blastomere close over the injured surface these partial embryos are rounded in form and many of the one-half larvæ resemble superficially whole larvæ of half size, but in no case are they complete. When the anterior or posterior quadrants of the 4-cell stage are killed nothing even remotely resembling a normal larva is ever produced. My results are therefore directly opposed to those of Driesch and they agree in all essential respects with those of Chabry.

The partial embryos and larvæ obtained in these experiments may be classified as right or left, anterior or posterior, dorsal or ventral, or composite forms. Furthermore they may be known as half, quarter, eighth, sixteenth, etc., embryos, according as they are produced from blastomeres of the 2, 4, 8, 16, etc., cell stages; however, the character of the embryo depends entirely upon the region from which the isolated blastomeres come and not upon the number of such blastomeres.

1. *Right or Left Half Embryos (Figs. 13–33, 36–46).*

a. Cleavage.

When the right or left half of an egg is injured in the 2, 4 or 8-cell stage, the other half continues to segment in a normal manner, provided it was not also injured. I have traced the cell-lineage of these right or left half embryos up to the eighth generation of cleavage cells (the 112-cell stage of normal eggs), while I have determined the lineage of many individual cells as late as the ninth or tenth generation (218–360 cell-stage). The cell-lineage of these half embryos is essentially like the right or left half of a normal egg, except that the direction of division and consequently the position and size of some of the blastomeres may be slightly altered.

This alteration in the direction of cleavage is most evident in cases where the egg was injured in the 2-cell stage, and it is probably due to the fact that the uninjured blastomere in such cases becomes nearly spherical in shape, and does not remain hemispherical as in the normal egg. Owing to this fact the median pole of certain cleavage spindles, *i. e.*, the one next to the original median plane, is shifted toward the middle of that plane. The resulting mass of cells is, therefore, more nearly spherical than in the half of a normal embryo. (Figs. 13–20.) If the injury occurs in the 4-cell stage or later, the change in the direction of the early cleavages is not so evident as when it takes place in the 2-cell stage. In case one of the blastomeres was injured at the close of the first cleavage, the direction of the karyokinetic spindles of the second and third cleavages are entirely normal, since in both these cases they lie parallel with the first cleavage plane, Fig. 13; but in the fourth cleavage in which one pole of the spindles lies nearer that plane than the other, the median pole is shifted toward the middle of that plane and consequently the cells formed along the median plane come into closer contact with one another and the cell aggregate is more nearly spherical than in the right or left half of a normal 16-cell stage. (Figs. 14, 15, 21, 22.). These results entirely agree with those of Chabry and Crampton.

The fifth cleavage of the right or left half embryo is also like the normal except in the direction of a few of the divisions; *e. g.*, Fig. 16 is nearly normal but in Fig. 17 the division of the cell

DEVELOPMENT OF RIGHT BLASTOMERE OF 2-CELL STAGE.

Figs. 13–20. Successive stages in the development of the same right half embryo, the left blastomere having been injured in the 2-cell stage; drawn at intervals of about five minutes. Here and elsewhere the yellow protoplasm is indicated by coarse stipples.

Fig. 13. Right half of 8-cell stage, posterior view. A small amount of yellow protoplasm surrounds the nucleus of the ectoderm cell $b^{4.2}$. The position of the cells shows that the ventral ends of the third cleavage spindles diverged from the first cleavage plane in the posterior quadrant and converged toward that plane in the anterior quadrant, just as in the normal egg. (See Conklin, '05[1].)

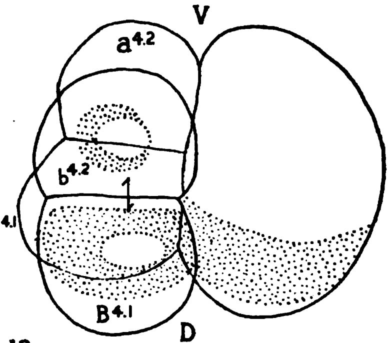
Fig. 14. Right half of 16-cell stage, anterior view. The yellow crescent is seen through the cell $B^{5.1}$. In the normal egg of this stage the cells $A^{5.1}$ and $a^{5.3}$ lie more nearly in front of the cells $A^{5.2}$ and $a^{5.4}$.

Fig. 15. Same stage as preceding posterior view. In normal eggs the cells $B^{5.2}$ and $b^{5.4}$ lie nearly behind the cells $B^{5.1}$ and $b^{5.3}$ and not on their median side.

Fig. 16. Right half of 30-cell stage, dorsal view. $A^{6.2}$ and $A^{6.4}$ are cells of the gray crescent; $B^{5.2}$ and $B^{6.2}$, cells of the yellow crescent.

Fig. 17. Right half of 34-cell stage, posterior view. In normal eggs the cell $B^{6.4}$ lies on the lateral border of $B^{6.3}$.

Fig. 18. Same stage as preceding, dorsal view. The cell $B^{6.1}$ normally lies between $B^{6.3}$ and $A^{6.1}$.



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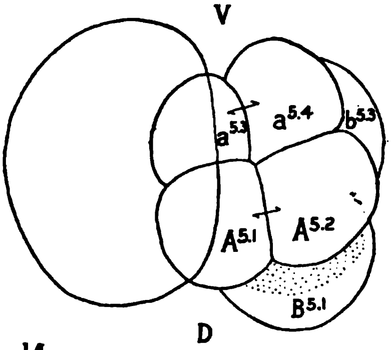

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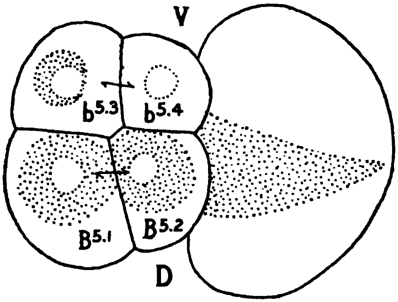

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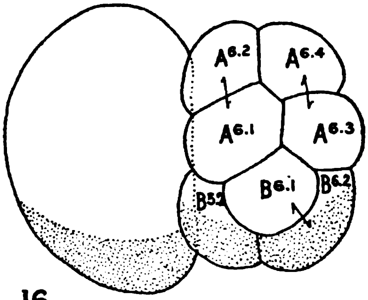

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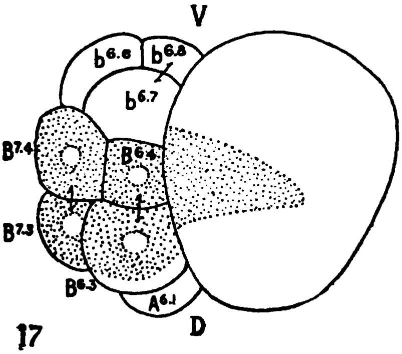

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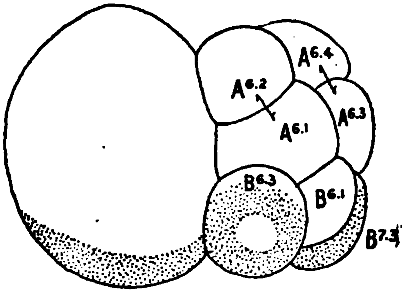

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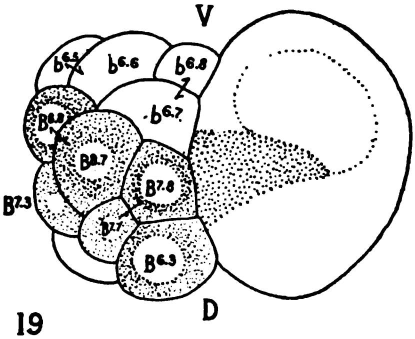
DEVELOPMENT OF RIGHT BLASTOMERE OF THE 2-CELL STAGE; ALSO OF RIGHT AND LEFT BLASTOMERES OF THE 4-CELL STAGE.

Figs. 19, 20. Same embryo as that shown in Figs. 13–18. Fig. 19. Right half of 46-cell stage, posterior view; the yellow crescent cells are not quite normal in position. Fig. 20. Right half of 48-cell stage, dorsal view. The caudal endoderm cells ($B^{7.1}$ and $B^{7.2}$) have been shoved away from the median plane by the cell $B^{7.5}$.

Figs. 21, 22. Fixed and stained preparations of half embryos in the 16-cell stage. Fig. 21. Right half embryo, posterior view. Fig. 22. Left half embryo, ventral-posterior view.

Figs. 23, 24. Successive stages of one and the same half embryo, the left half having been injured in the 4-cell stage, dorsal view. Fig. 23. Right half of 16-cell stage. Fig. 24. Right half of 32-cell stage. The cleavage is like the right half of a normal egg in every respect.



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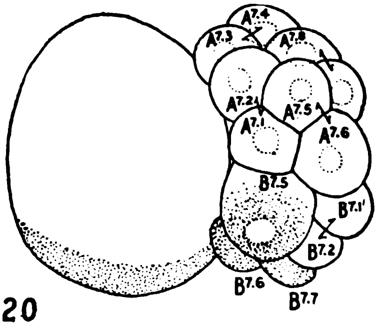

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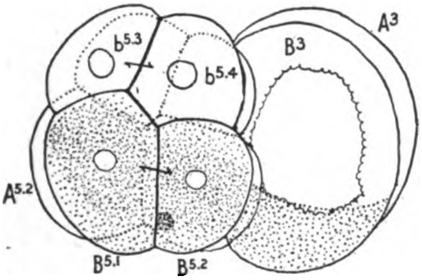

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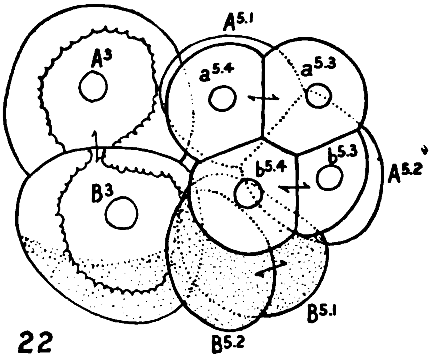

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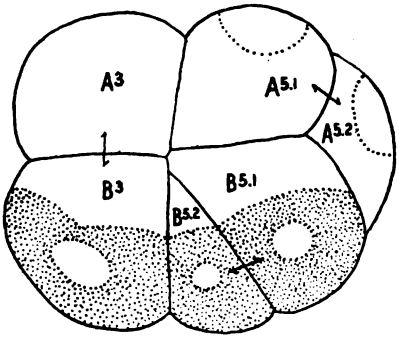

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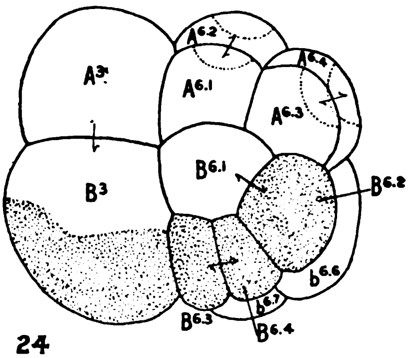

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$B^{5.2}$ into $B^{6.3}$ and $B^{6.4}$ is almost at right angles to its normal direction. In other cases, as is shown in Fig. 24, this cleavage is normal in direction, and I am, therefore, of the opinion that the condition shown in Fig. 17 and the later stage of this same egg shown in Fig. 19 may be due to some slight injury to the developing half of this egg. In Fig. 18, which is a dorsal view of the same egg in the same stage as Fig. 17, the cells $A^{6.2}$ and $A^{6.4}$ have moved in toward the median plane as compared with Fig. 16, though in this respect, also, the corresponding stage shown in Fig. 24 is quite normal. This shifting of the anterior dorsal cells toward the median plane is shown again at the next cleavage (the sixth), of this egg. (Fig. 20.)

The seventh cleavage, which is shown in Figs. 25 and 26, is also normal except for the direction of a few of the divisions. The cells which constitute the yellow and gray crescents are in all respects like the right half of a normal egg. However the position of the cells $A^{7.1}$ and $A^{7.2}$, Fig. 25, and the direction of division in several of the ectoderm cells shown in Fig. 26 are not quite normal.

In conclusion therefore it may be said that the cleavage of one of the blastomeres of the 2-cell stage or of the right or left blastomeres of the 4-cell stage, is like that of the corresponding half of a normal egg, except in minor details. Even these minor differences are not always present and when they are they do not alter the localization of the oöplasmic substances. In every case the distribution of the yellow, the gray and the clear substances to the different blastomeres is the same as in the right or left half of a normal egg; the cells of the yellow crescent, for example, form only the right or left half of a normal crescent, and the same is true of the gray crescent and of the other substances of the egg. Even the small amount of yellow protoplasm which is found around the nuclei of the posterior ectoderm cells $b^{4.2}$, Fig. 13, is perfectly normal in its occurrence and subsequent distribution.

I have elsewhere ('05^1) shown that the localization of different oöplasmic substances in the ascidian egg precedes cleavage and that cleavage and localization are here relatively independent of each other; these experiments show that in both cleavage and localization the development of the right or left half of an ascidian egg is a "mosaic work," for the slight amount of regulation, which is manifested in the changes in the direction of certain cleavages, and the consequent closing of the embryo in no way alters the histological character of the cleavage cells nor their developmental tendencies.

b. Gastrulation.

In the development of the right or left half of an egg the process of gastrulation sometimes occurs in an unusual manner. The most frequent modification of the normal process is that shown in Figs. 27, 29, 30, where the endoderm cells are not infolded but come to protrude above the level of the other cells, thus forming exogastrulæ. In later stages these endoderm cells must become infolded for it is a rare thing to see exogastrulæ or any indication of an original evagination of endoderm cells in any of the cultures of older embryos. By what process these exogastrulæ right themselves I have not been able to observe, but I think it probable that this like normal gastrulation is accomplished by overgrowth of the ectoderm cells and change of shape of the endoderm cells.

Sometimes when the endoderm cells are evaginated other portions of the blastula wall invaginate. In this way false gastrulæ may arise in which the infolded cells are not endodermal but ectodermal, as is clearly shown by their histological structure. (Fig. 63.)

While some embryos in the gastrula stage show such abnormalities as those which have just been described in other cases the gastrula is strictly a half one, as is shown in Fig. 31, and it seems to me probable that exogastrulæ or false gastrulæ only arise when the surviving half of the egg has been slightly injured. These half gastrulæ contain just one-half of all the cells of the normal gastrula and the position of the various cells and organ bases is essentially like that which occurs in the right or left half of a normal gastrula; the cells of the yellow crescent lie along one side only of the blastopore groove; the neural plate and chorda cells each form half of the arc which is normally present in the anterior lip of the blastopore, while the closing of the open side of the gastrula, which is turned toward the injured cell, is chiefly accomplished by the overgrowth of the ectoderm cells of the ventral side. (Fig. 31.)

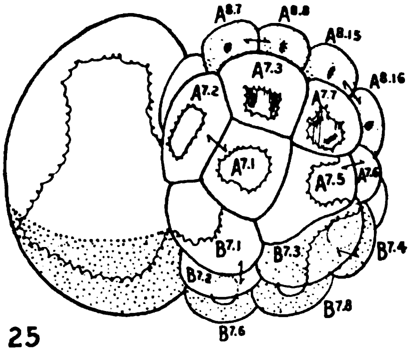
Except, therefore, for this tendency of the cells along the injured side to come together, these half gastrulæ are strictly partial and the gastrulation no less than the cleavage may be regarded as an illustration of mosaic development.

RIGHT OR LEFT HALF EMBRYOS; 64-CELLS TO GASTRULA.

Figs. 25, 26. Fixed and stained half embryos; spurted in the 2-cell stage and fixed 2 hours later. Fig. 25. Right half of 64–76-cell stage, dorsal view. The neural plate cells ($A^{8.7}$, $A^{8.8}$, $A^{8.15}$, $A^{8.16}$) have just divided, the chorda cells ($A^{7.3}$, $A^{7.7}$) are dividing. The position of the cells $A^{7.1}$, $A^{7.2}$ is slightly abnormal. (*v.* Fig. 6.) Fig. 26. Left half of 64–76-cell stage, ventral-posterior view.

Figs. 27, 28. Right half of embryo in about 180-cell stage; spurted in the 4-cell stage and fixed 2½ hours later. Fig. 27. Dorsal view; the large endoderm cells lie above the level of the other cells and form an exogastrula; some of the yellow cells (stippled) still lie at the surface while others are covered by endoderm cells. Fig. 28. Ventral view of similar embryo.

Figs. 29, 30. Living right half embryos, dorsal view, showing the endoderm cells forming exogastrulæ and the yellow crescent cells at the surface.



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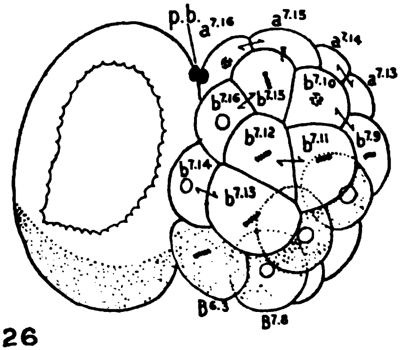

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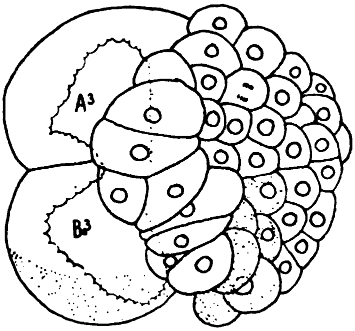

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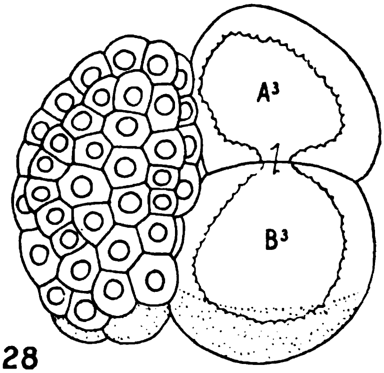

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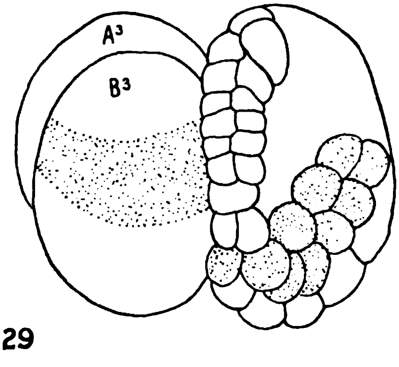

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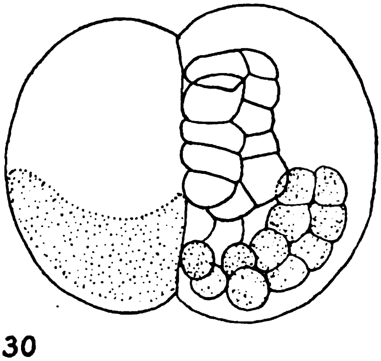

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RIGHT OR LEFT HALF OR THREE-QUARTER EMBRYOS; GASTRULA TO TADPOLE. DRAWN FROM FIXED AND STAINED MATERIAL.

Fig. 31. Right half gastrula of about 220-cell stage; spurted in the 4-cell stage and fixed 3 hours later. The neural plate, chorda and mesoderm cells are present only on the right side and in their normal positions and numbers.

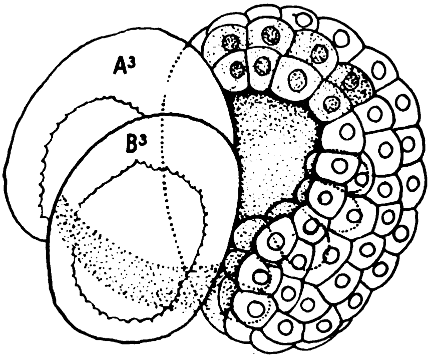
Fig. 32. Left half of young tadpole, dorsal view; spurted in the 4-cell stage, fixed 5 hours later. The notochord is normal except for size and number of cells; the muscle and mesenchyme cells are present only on one side; the neural plate is abnormal in form but not in position.

Fig. 33. Right half of young tadpole, dorsal view; spurted in the 4-cell stage, fixed 4½ hours later (slightly younger stage than Fig. 32). The notochord consists of a small number of cells which are interdigitating; muscle cells and mesenchyme lie on the right side of the notochord, but not on the left, though the muscle cells have begun to grow around to the left side; the neural plate is normal in position but not in form.

Fig. 34. Right-posterior three-quarter embryo, from the right side. The left anterior cells ($\mathrm{A}^{4.1}$, $\mathrm{a}^{4.2}$) were killed in the 8-cell stage and the embryo fixed 5 hours later. The posterior half of the embryo is normal, but the left half of the anterior part is lacking and the neural plate is abnormal and has not formed a tube though sense spots are present.

Fig. 35. Left-anterior three-quarter embryo, dorsal view; the right posterior quadrant (B^{3}) was killed in the 4-cell stage and the embryo fixed 6 hours later. The anterior half of the embryo is entirely normal. The muscle cells are lacking on the right side though they have begun to grow around the hinder end of the notochord. The posterior portion of the trunk mesenchyme is found only on the left side, but its anterior portion, which is derived from the cells $A^{7.6}$ and $\mathrm{A}^{7.6}$ (Fig. 6) of the anterior quadrants is present on both sides. In the region of the injured cell the notochord and neural tube are curved away from that cell.

Fig. 36. Left half embryo, from left side; spurted in the 4-cell stage, fixed 6 hours later. The dorsal lip of the blastopore is being overgrown by the ventral (posterior) lip. Muscle cells and mesenchyme are found only on the left side. The neural plate is abnormally folded, but still open; sense spots are present.



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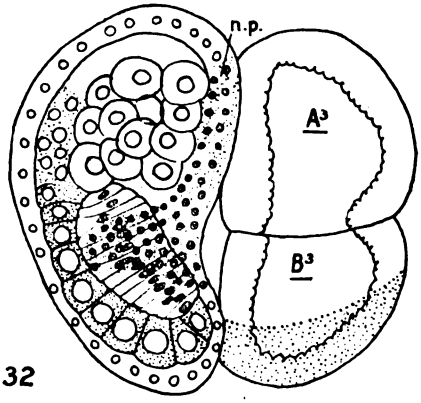

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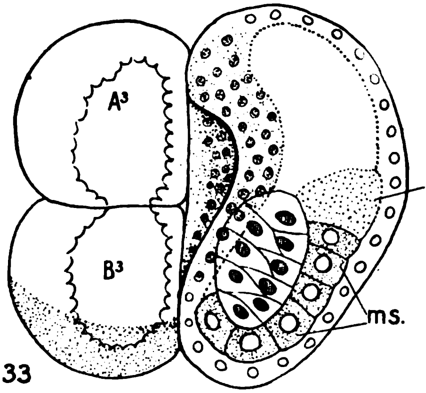

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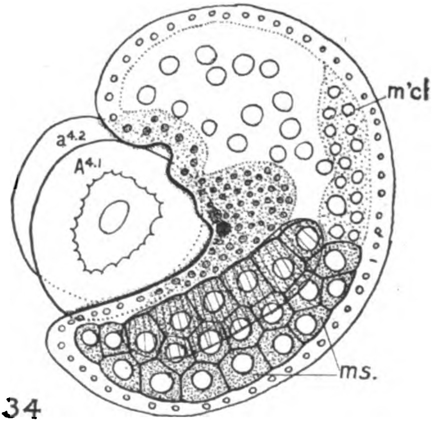

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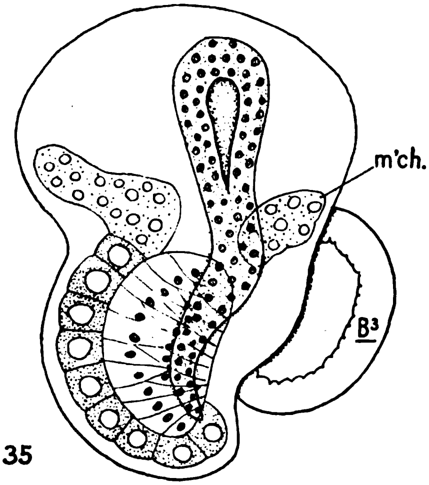

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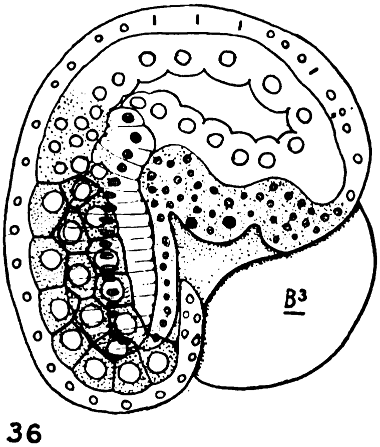

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c. Formation of Larva.

A considerably later stage in the development of the half embryo is shown in Figs. 32, 33 and 36 (Figs. 34 and 35 are three-quarter embryos and will be described later); of these stages Fig. 33 is the youngest and Fig. 36 the oldest. In all of these figures the blastopore has already closed and the chorda cells have given rise to a fusiform notochord, which lies in the posterior half of the embryo. The blastopore closes chiefly by the posterior growth of the dorsal (anterior) lip, as in the normal gastrula. With the formation of the notochord the posterior half of the embryo becomes elongated and narrower than the anterior half and the developing tail bends around toward the injured side. (Figs. 32, 33.)

The anterior half remains large, the posterior half becomes long and narrow; the latter portion contains the notochord and muscle cells, the former the gastral endoderm, mesenchyme and most of the neural plate. The general superficial appearance of an embryo of this stage is very similar to a normal one, but a more detailed study shows many differences.

(*1*) *Neural Plate.* The neural plate occupies in the main its normal position, that is, it lies along the first cleavage plane on the dorsal side, next to the injured cell. In this position the plate becomes folded and ultimately comes to contain a vesicle (the sense vesicle) though the steps by which this vesicle is formed are always irregular and abnormal. (Figs. 36–40.) The anterior portion of the plate is usually doubled over posteriorly while the posterior portion is folded forward (Figs. 36, 39, 40) and in this way a vesicle is finally formed.

The tail of the embryo grows around toward the injured side so that the concave side of the embryo is median or dorsal, the convex side being lateral or ventral. In the younger, normal larvæ the concave side is ventral, the convex dorsal. In these half larvæ the nerve plate lies along the concave side, a condition which is the reverse of what is found in the normal larva. (*cf.* Figs. 12 and 36.) In the older half larvæ there is almost always found one or more pigmented sense spots in the neural plate or sense vesicle. (Figs. 36–40, 45, 46.) These pigment spots appear within cells of the neural plate and, as I am well convinced, always within definite cells, though owing to the abnormal foldings of the neural plate they do not always occupy exactly the same positions. Furthermore these sense spots may be more numerous than in the normal larva, as shown in Figs. 45 and 46, probably owing to the fact that the cells which form the pigment and which normally lie on the margins of the neural plate do not come together to form two spots as in normal larvæ, but remain separated so that several such spots are formed.

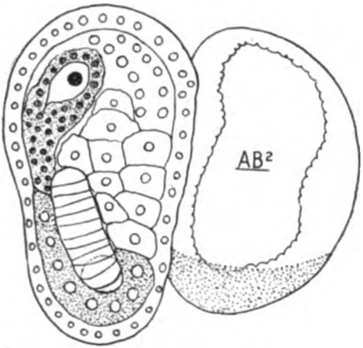
(2) *Notochord.* The chorda cells grow back into the posterior half of the embryo and the cells here interdigitate in the normal manner, finally forming a linear series of cells. (Figs. 32–46.) The notochord, which is at first relatively short and thick, Fig. 33, becomes later very much longer and more slender, Fig. 40, and in all respects it has the appearance of a normal notochord, save that it evidently contains a smaller number of cells. The position of the notochord of the half larva is always slightly abnormal; it never lies along the original median plane (first cleavage) as in normal larvæ, but its anterior end is diverted away from that plane and toward the lateral border of the larva. (Figs. 32, 33, 37, 41.) This position is that which the chorda cells, which arise in the anterior lip of the blastopore and which grow posteriorly around the margin of the blastopore, would naturally assume. (*cf.* Figs. 31 and 33.) What it is which causes the chorda cells to interdigitate in their characteristic manner is a question difficult to answer; it certainly is not dependent upon the crowding together of chorda cells from the right and left sides since it occurs normally when the cells of one side only are present; on the other hand it must depend upon a certain amount of lateral compression of the chorda cells since it occurs very rarely if at all in the anterior half larvæ in which the ectoderm and mesoderm of the tail are lacking.

(3) *Muscles and Mesenchyme.* In these right or left half embryos and larvæ the muscle and mesenchyme cells are present on one side of the notochord; here they occupy their normal positions, the muscle cells giving rise to three rows of cells along the lateral border of the notochord and the mesenchyme forming a group of small cells anterior to the muscle rows. (Figs. 32, 33, 36.) In later stages the muscle cells slowly extend over to the side of the tail on which they were originally lacking; this takes place especially at the hinder end of the tail, the overgrowth taking place around the end of the notochord and over its ventral side. In this way the right or left half embryo or larva tends to become complete, but I have never seen a case in which three rows of muscle

cells were found on both sides of the notochord. Indeed, I am not at all sure that this extension of the muscle cells around the end of the notochord is accompanied by any increase whatever in the number of muscle cells or in the number of rows of cells. The latest stage in which I can positively identify the three rows of muscle cells is shown in Fig. 36. In this larva the muscle rows lie nearer the ventral side than in normal larvæ (see Fig. 12), and they are evidently extending over the ventral surface toward the opposite side. In later stages the muscle cells become much elongated, but I have not been able to determine the number of rows present. I have found it still more difficult to decide whether the trunk mesenchyme ever extends over to the side on which it was originally lacking, but I believe that this takes place only to a limited extent, if at all, and that Chabry was right when he affirmed that only one atrial invagination is formed in these right or left half embryos.

2. *Three-Quarter Embryos (Figs. 34–35).*

In connection with the right or left half embryos I shall here consider three-quarter embryos, which, of course, include the whole of the right or left half. Two such embryos are shown in Figs. 34 and 35. In the former the left anterior quadrant was killed in the 8-cell stage; in the latter the right posterior quadrant in the 4-cell stage. The embryo in which the cells of the anterior quadrants were uninjured (Fig. 35) is perfectly normal in its anterior half; its posterior half, however, lacks those parts which would have developed from the cell which was injured. This embryo is younger than the one shown in Fig. 34 and no sense spots are present, but the sense vesicle is closing in a normal manner. This figure well shows that a part of the trunk mesenchyme is derived from the anterior quadrants, and indeed from the pair of cells $A^{7.6}$, Fig. 6, while a portion of it comes from the posterior quadrants, as may be seen by comparing the right and left sides of Fig. 35. The muscle cells are entirely lacking on the right side, the substance which would have formed them being located in the injured cell B^{3}; they are shown growing around the end of the notochord as in the half embryo shown in Fig. 33. The notochord and nerve tube are apparently full sized, which is explained by the fact that they come from the anterior quadrants, but owing to the lack of the right side of the tail they are somewhat distorted in form.



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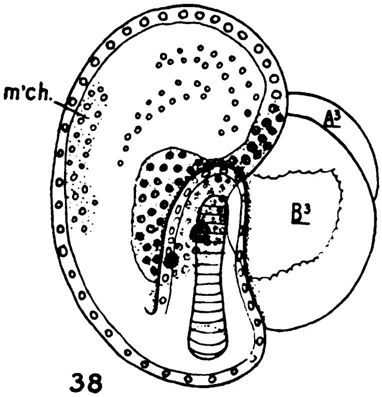

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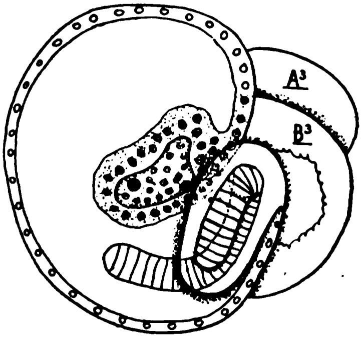

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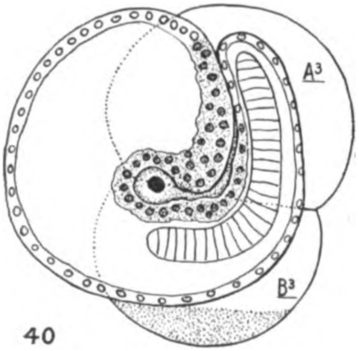

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Right or Left Half Larvæ. Fixed and Stained Material.

Figs. 37–40. Four half larvæ from eggs which were spurted in the 2-cell or 4-cell stage and fixed 22 hours later. These larvæ are still within the egg membranes though at a corresponding age normal larvæ are undergoing metamorphosis.

Fig. 37. Right half larva, ventral view. The tail which is elongated is turned down toward the dorsal side; the sense vesicle also lies on the dorsal side and is here seen through the embryo. The muscle cells are chiefly on one side of the notochord but have grown over to the other side at the posterior end.

Fig. 38. Left half larva, dorsal view. The neural plate with sense spots is partly covered by the end of the tail. The mesenchyme is found only on the left side.

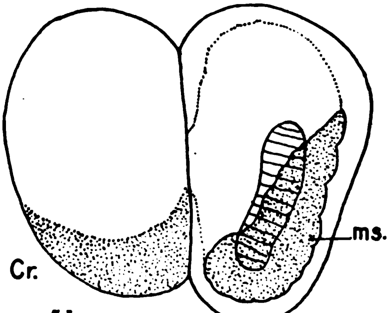
Fig. 39. Left half larva from the left side. The neural plate is folded so as to form a nearly closed sense vesicle, in which are two sense spots.

Fig. 40. Left half larva viewed from the left side. The neural plate is partially closed, but is abnormal in form. In all of these larvæ the neural plate lies on the concave side.

RIGHT OR LEFT HALF LARVAE DRAWN FROM LIVING SPECIMENS FROM 12 HOURS (FIGS. 41, 42) TO 20 HOURS (FIGS. 45, 46) AFTER THE INJURY OF ONE OF THE FIRST TWO BLASTOMERES.

Fig. 41. Posterior-dorsal view. Fig. 42. Same embryo, posterior ventral view. In both these figures the muscle cells are found chiefly on one side of the notochord, but they have grown over to the opposite side at the end of the tail. Fig. 43. Right half larva from right side. Fig. 44. Left half larva from left dorsal side. The yellow crescent on the injured blastomere apparently occupies different positions with respect to the larva in these two figures, but it is by no means certain that the convex side of the larva is morphologically the same in the two figures.

Figs. 45, 46. Two views of one and the same left half larva. Fig. 45, from the dorsal side; Fig. 46, from the left side, showing two sense spots on the dorsal and two on the ventral sides. The neural plate is continuous between these spots on the side next to the injured blastomere.



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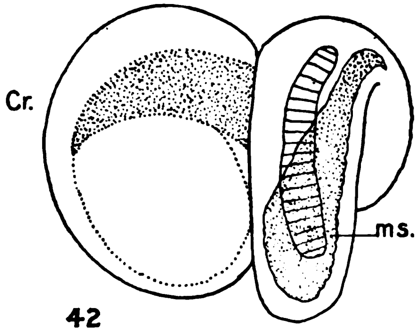

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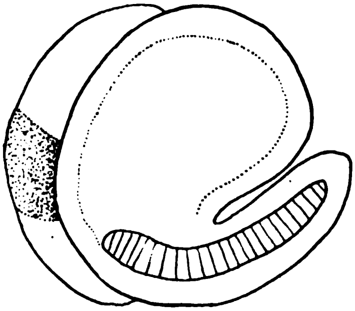

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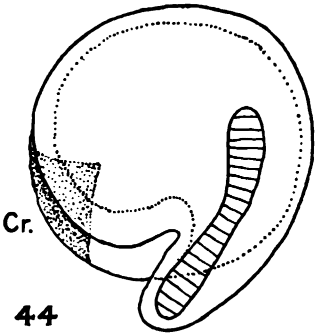

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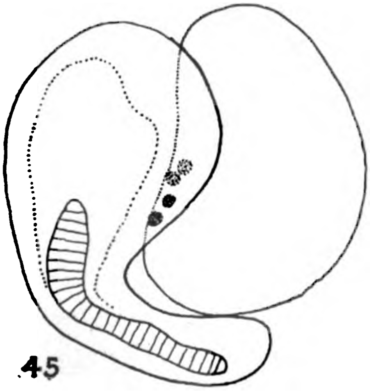

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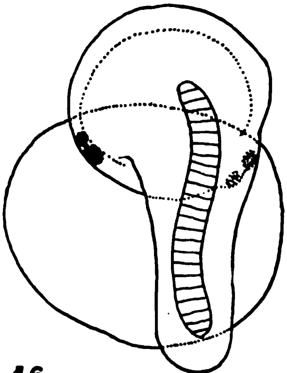

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In Fig. 34 the left anterior quadrant was killed and the posterior portion of this embryo is normal save only for the fact that the notochord and nerve tube are smaller than usual, which is explained by the fact that the substance of these organs is derived from the anterior quadrants; three rows of muscle cells are found on both sides of the tail. The anterior half of this embryo, on the other hand, is quite defective; the neural plate is irregularly folded and has not formed a sense vesicle, although sense spots are present.

I have seen and studied many three-quarter embryos similar to those shown in Figs. 34 and 35 and they all show, as do the right and left half embryos, that where part of the substance which would normally form an organ is destroyed the organ which develops is defective, whereas if all or any organ-forming substance is lacking the organ to which it would normally give rise is also lacking.

So far as I have observed these partial larvæ never escape from the egg membrane, and in this my observations accord with those of Chabry and Driesch, and although I have kept them alive until a period after the normal larvæ have undergone metamorphosis I have never observed this transformation in them.

In conclusion then I find that the cleavage and gastrulation of these half or three-quarter embryos is partial and the resulting larva incomplete although the notochord is well formed and there is a tendency on the part of some of the cells to grow over and close up the open side of the larva. However, this regulation never leads to the formation of a complete larva; the neural plate may close, but it forms an abnormal sense vesicle; at the end of the tail the muscle cells extend over toward the injured side, but they do not form three rows of cells on each side of the notochord as in the normal larva; the mesenchyme likewise does not develop along the injured side and it is probable that only one atrial invagination is formed.

Furthermore *not a single cleavage cell nor any one of the oöplasmic substances ever gives rise to parts or organs which it would not normally produce; the notochord, for example, invariably comes from the chorda cells, the sense vesicle from the neural plate cells and both these structures from the material of the gray crescent; the muscles always come from the muscle cells and these from the substance of the yellow crescent; the ectoderm, from the ectoderm cells and ultimately from the clear protoplasm; the endoderm, from the* *endoderm cells and these from the deep gray material of the egg.* In spite therefore of the regulation which is apparent in the closing of the open side of the embryo, and in the formation of a whole notochord and of an imperfect sense vesicle, the various oöplasmic substances of the unsegmented egg and of the different blastomeres are not totipotent but each shows in these experiments, as well as in normal development, that it is differentiated to give rise to one, and only one, particular kind of tissue.

3. *Anterior Half Embryos (Figs. 47–52).*

The anterior and posterior half embryos show even more clearly than do the lateral ones the mosaic character of the development of these eggs. When the posterior half of an egg is killed in the 4-cell or 8-cell stage the anterior half continues to develop as if the posterior half were still living. The cleavage is in all respects like that of the anterior half of a normal egg; the gastrulation is essentially the same, but the later development is modified in many important particulars.

Figs. 47 and 48 are ventral and dorsal views, respectively, of one and the same living embryo of the 76-cell stage, in which the posterior dorsal cells, $B^{4.1}$, containing the yellow crescent, were killed in the 8-cell stage. None of the cells of the ventral hemisphere were injured and consequently the cleavage of these cells is quite normal; thirty-two ectoderm cells are present, all of which have entirely normal positions, shapes and sizes. (*cf.* Figs. 5 and 47.) The anterior half of the dorsal hemisphere is also entirely normal (*cf.* Figs. 6 and 48); eight chorda cells are shown forming an arc which bounds anteriorly the six endoderm cells and which is flanked on each side by the anterior mesenchyme cell, $A^{7.6}$. The number, size and position of each and all of these cells is the exact counterpart of what is found in the normal embryo, and, although the outlines of the neural plate cells were so indistinct in the living specimen from which this figure was made that I could not draw them, there is every reason to suppose that these cells like all the others in this embryo conform to the normal type.

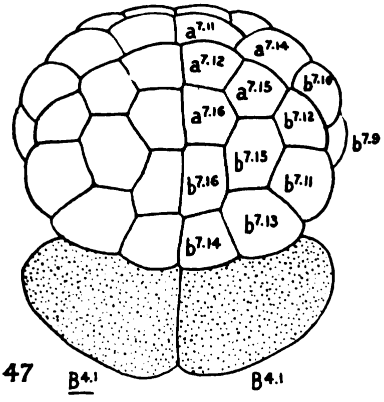
In the posterior half of the dorsal hemisphere all the parts which would have developed from the cells $B^{4.1}$ and $\mathit{B}^{4.1}$ are entirely lacking; there are neither mesenchyme, caudal endoderm.

ANTERIOR HALF AND THREE-QUARTER EMBRYOS; 76 CELLS TO METAMORPHOSIS.

Figs. 47, 48. Anterior-ventral three-quarter embryo of the 76-cell stage (*v.* Figs. 5 and 6); the dorsal posterior cells $B^{4.1}$, containing all of the yellow crescent, were killed in the 8-cell stage. The ventral ectoderm cells (Fig. 47) are quite normal both in position and number (*cf.* Figs. 5 and 47); the anterior dorsal cells are also normal, but the posterior dorsal cells (muscle, mesenchyme and caudal endoderm) are entirely lacking. (*cf.* Figs. 6 and 48.)

Figs. 49–51. Three views of one and the same anterior half embryo of about the 250-cell stage; spurted in the 4-cell stage and fixed 2 hours later. Fig. 49. Dorsal view, superficial focus, showing the neural plate. Fig. 50. Dorsal view, deeper focus, showing two rows of chorda cells besides several ectoderm and endoderm cells. Fig. 51. Dorsal view, still deeper focus, showing the cells of the ventral ectoderm.

Fig. 52. Anterior half embryo, dorsal view. Spurted in the 4-cell stage, fixed 22 hours later. The yellow crescent is plainly visible in the injured cells. Sense spots are present but the neural plate never forms a tube. The chorda cells lie in a heap at the left side. There is no trace of muscle substance or of a tail in this anterior half embryo. This embryo is from the same experiment as Figs. 37–40; normal larvæ of this stage are undergoing metamorphosis.



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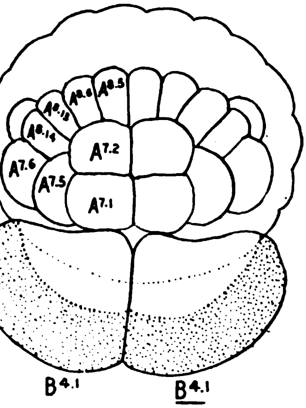

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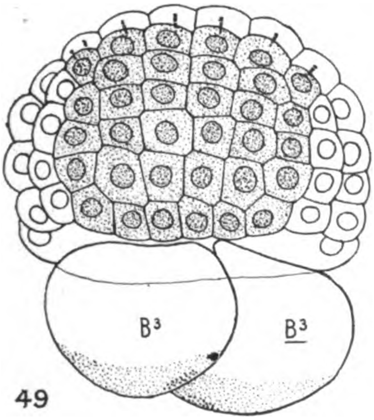

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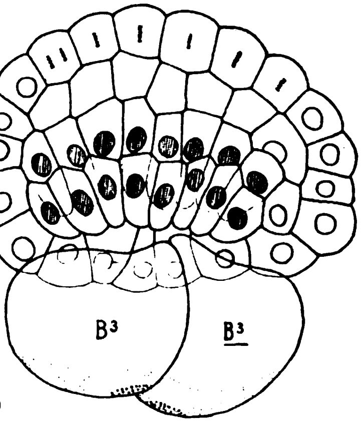

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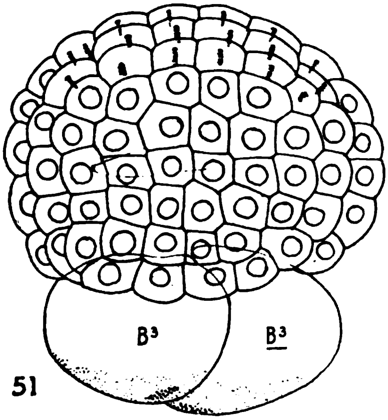

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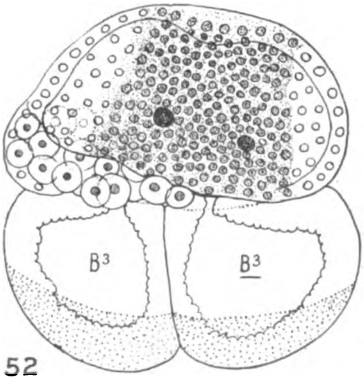

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nor muscle cells. Unfortunately this particular embryo was not followed through the various stages of development until it gave rise to a larva and none of the older stages which I have studied have shown precisely this type of injury, *i. e.*, the destruction of the yellow crescent without injury to the ectoderm cells of the posterior half.

In many other cases which I have seen all of the posterior half of the egg was injured in the 4-cell stage. I have followed the development of the surviving anterior halves of such eggs as late as the stage of the metamorphosis of the normal larvæ; the development of such blastomeres is always partial. Figs. 49, 50 and 51 represent three views of one and the same anterior half embryo of about the 250-cell stage; in all the figures the embryo is viewed from the dorsal side, but in Fig. 49 the focus is high and only the ectoderm and neural plate cells of the dorsal surface are shown; Fig. 50 is a median optical section showing chorda and endoderm cells surrounded on the anterior side by ectoderm; Fig. 51 represents the ectoderm of the ventral surface which is visible at a deep focus. This half embryo is exactly like the anterior half of a normal one in the formation of the neural plate, the chorda plate, the general ectoderm and gastral endoderm, in the overgrowth of the dorsal lip of the blastopore, even in the position, shape and size of the individual cells. (*cf.* Figs. 9 and 10.)

Finally in Fig. 52 there is represented an anterior half embryo 22 hours after the posterior cells were killed, and at a stage when normal larvæ of corresponding age have already undergone metamorphosis. The ectoderm has not yet inclosed the embryo on the side next the injured cells, and this rarely happens in anterior or posterior half embryos. The neural plate has not rolled up nor invaginated to form a tube, though it is slightly depressed along its median line; two sense spots are present though there is no sense vesicle. The large rounded chorda cells are irregularly scattered along the posterior border of the embryo, where they project beyond the ectoderm; they never form a notochord. There is no trace of yellow crescent substance nor of muscle cells in these anterior larvæ and no indication whatever of a tail. They are, therefore, altogether unlike the normal larvæ and they afford complete and convincing evidence that the anterior blastomeres of the ascidian egg are not totipotent but rather that the development is a mosaic work.

4. *Posterior Half Embryos* (*Figs. 53–58*).

All that has been said of the mosaic-like development of the anterior half of the egg is equally true of the posterior half. The cleavage progresses in normal fashion up to the time of the closure of the blastopore. Figs. 53 and 54 represent posterior half embryos of the 32-cell and 76-cell stages, respectively. The former is entirely normal and the latter is normal in all respects save that a single pair of cells, $B^{8.6}$, is larger than in the normal embryo. The clear, the yellow and the gray substances of the egg are distributed exactly as in the posterior half of a normal embryo. The clear ectoderm cells lie on the ventral side and only two of them appear in the dorsal view shown in Fig. 54 (the two clear cells at the posterior pole). In Fig. 53 the gray endoplasm is contained in two cells ($B^{6.1}$) and in Fig. 54, in four ($B^{7.1}$, $B^{7.2}$); these cells give rise to the strand of caudal endoderm. The yellow crescent consists at the 32-cell stage of a single arc of yellow cells (Fig. 53) which then, by division, become a double arc of fourteen cells (Fig. 54); the inner arc consists of eight mesenchyme cells and the outer of six muscle cells. In all these respects these posterior half embryos are entirely like the posterior half of a normal embryo.

But while the pregastrular stages of these posterior half embryos are like the normal, the gastrulæ and later stages show many interesting modifications. Figs. 55, 56, 57 are three views of one and the same posterior half embryo, the normal embryos of the same stage being young tadpoles like Fig. 11. In all of these figures the embryo is viewed from the dorsal side; Fig. 55 shows the ectoderm cells which cover the dorsal surface; Fig. 56, the muscle cells which lie below the ectoderm on the dorsal side; Fig. 57 is an optical section at a still deeper level showing the caudal endoderm and mesenchyme. Fig. 58 is another posterior half embryo of similar age seen from the ventral side, showing the yellow mesoderm cells on each side of the caudal endoderm.

The gastrulation occurs between the stages shown in Figs. 54 and 55. The caudal endoderm and the surrounding arc of mesenchyme, shown in Fig. 54, invaginates; the muscle cells come to lie above (dorsal to) the mesenchyme cells and finally the latter are overgrown by the ectoderm in the manner shown in Fig. 8. In normal embryos the posterior part of the blastopore is closed chiefly by the growth of the anterior lip; in the latter stages of

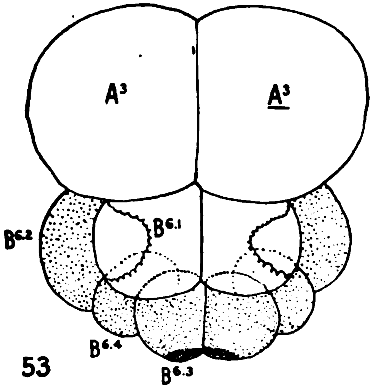
POSTERIOR HALF EMBRYOS; 32 CELLS TO TADPOLE STAGE. FIXED AND STAINED PREPARATIONS.

Fig. 53. Posterior half of 32-cell stage, dorsal view. The cleavage is altogether normal. Spurted in the 4-cell stage, fixed 1 hour later.

Fig. 54. Posterior half of 76-cell stage (*cf.* Fig. 6); spurted in the 4-cell stage, fixed 2 hours later. Two rows of yellow crescent cells are present, the inner being mesenchyme, the outer muscle cells; the anterior pair of mesenchyme cells ($B^{8.6}$) are larger than normal. There are two pairs of caudal endoderm cells ($B^{7.1}$ and $B^{7.2}$). A pair of ventral ectoderm cells is visible in the midline behind.

Figs. 55–57. Three views of one and the same embryo; spurted in the 4-cell stage, fixed 4 hours later, normal embryos being in the stage represented by Fig. 11. Fig. 55. Dorsal view of the superficial ectoderm. The notch in front represents the notch in the ventral lip of the blastopore. Fig. 56. Same view, deeper focus, showing the muscle cells beneath the ectoderm; these cells are continuous from side to side, there being no chorda in the midline. Fig. 57. Same view, still deeper focus, showing the double row of ventral endoderm cells in the midline, and on each side of this a mass of mesenchyme cells.

Fig. 58. Ventral view of posterior half embryo of the same stage as the preceding, showing the muscle and mesenchyme cells beneath the ectoderm and on each side of the strand of ventral endoderm.



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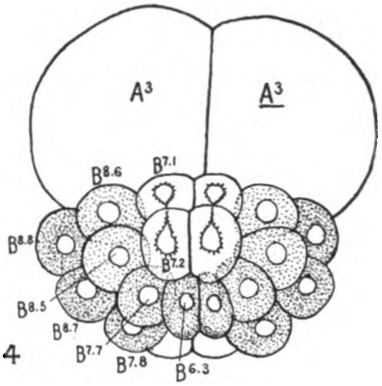

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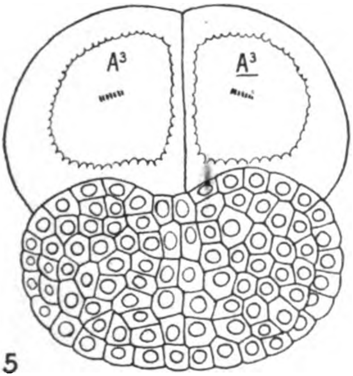

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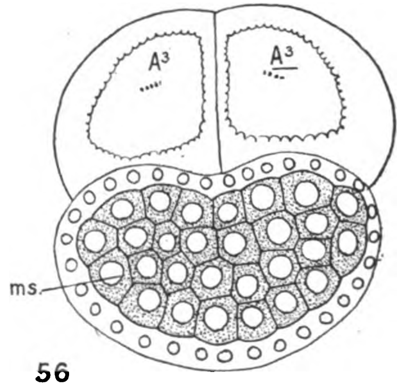

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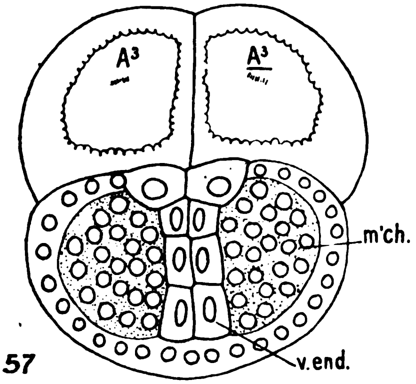

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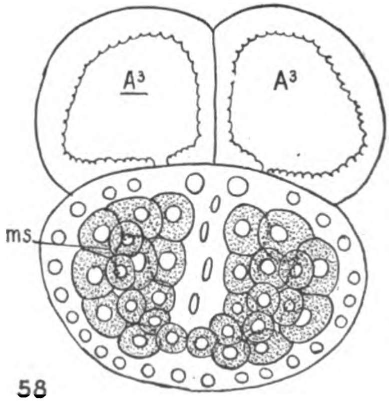

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gastrulation a blastopore groove is left in the posterior half of the embryo, on each side of which lie the muscle cells. (Fig. 9.) By the continued growth of the anterior lip this groove is shoved to the posterior end of the embryo and the rows of muscle cells are tilted up from an antero-posterior to a vertical position. Later, when the notochord is formed, the muscle cells come to lie alongside of it, thus forming the three rows of muscle cells on each side. Finally the ectoderm of the posterior lip of the blastopore, which has, up to this stage, formed a notch at the end of the blastopore groove, grows forward and reduces this groove to a minute pore.

Owing to the absence of the anterior lip of the blastopore, and of the notochord and the neural plate, the later stages in the development of these posterior half embryos is much altered. In the first place the blastopore groove and the muscle cells are not pushed to the posterior end of the embryo. Then the muscle cells on each side of the blastopore groove are not kept apart by the notochord but come into contact forming a continuous layer of muscle cells across the dorsal side. (Fig. 56.) The blastopore groove, therefore, disappears by the fusion of the lateral lips of the groove and the ectoderm cells grow over the whole dorsal surface; the only trace of the blastopore groove which is left is a slight notch in the anterior border of the embryo. (Figs. 55, 56.) The ectoderm never entirely incloses the posterior half embryo on the side next the injured cells, but the endoderm here comes to the surface as shown in Figs. 57 and 58.

No trace of notochord, neural plate nor sense spots ever appears in these posterior half embryos, and what is more remarkable a tail is never formed but the embryo always remains rounded in form, as shown in Figs. 55–58. It is quite evident that the elongation of the tail of the normal larva, together with the elongation of the individual muscle cells and perhaps also the arrangement of these cells in three rows on each side, is dependent upon the presence and elongation of the notochord. Perhaps one reason why a normal notochord is never formed in the anterior half embryo is due to the fact that the ectoderm does not completely inclose the embryo, so that the chorda cells in their growth crowd out of the open side and hence become free and scattered.

In conclusion, the study of anterior or posterior half embryos establishes in a most convincing manner the fact that the development of individual blastomeres of the ascidian egg is a mosaic work.

These blastomeres give rise only to those tissues and parts of an embryo which would come from them normally. Nothing even remotely resembling a complete normal larva is ever produced from the anterior or posterior quadrants of the egg.

5. *Quarter Embryos (Figs. 59–70).*

The development of individual blastomeres of the 4-cell stage furnishes additional confirmation of the mosaic theory as applied to ascidian eggs; in every instance individual blastomeres give rise only to those parts or organs which they would produce in normal embryos. Quarter embryos generally show more abnormalities and variations than half embryos,—probably owing to the more severe injury which they have suffered, which often affects the surviving quarter of the egg.

The cleavage of these quarter eggs is normal in every detail, save that the position of the cells is sometimes slightly altered; the rhythm of cleavage and the size and quality of the cells is the same as in the corresponding quarter of a normal egg. In Fig. 59, which corresponds to the 16-cell stage of the normal egg, each of the surviving quadrants has divided twice; in Fig. 60 the left posterior quadrant of a 44-cell stage is shown and in both of these figures the size, quality and position of the cells as well as the rhythm of division and the distribution of the different oöplasmic substances is entirely normal. Fig. 61, which is the right anterior quadrant of the 76-cell stage, is normal in every respect, save for the position of the endoderm cells which are here displaced toward the first cleavage plane. The mesoderm cells in the right posterior quadrant, shown in Fig. 62, are not normal in position; the two caudal endoderm cells (lying next the first cleavage plane) are, however, normal and the ectoderm cells are normal save that they show a tendency to grow inward at the first and second cleavage furrows and thus surround the embryo. In particular, attention should be directed to the yellow crescent and caudal endoderm cells in Fig. 60, and to the neural plate and chorda arcs in Fig. 61, which are similar in every respect to the quarter of a normal embryo at these stages.

I have already commented upon the fact that the quarter embryo shown in Fig. 63 is a "false gastrula" since the invaginated cells are ectodermal, probably neural plate cells, while the larger endo-

QUARTER EMBRYOS; 16 CELLS TO YOUNG TADPOLE STAGE. FIXED AND STAINED PREPARATIONS.

Fig. 59. Left anterior and right posterior (diagonal) quarter embryos of the 16-cell stage, ventral view.

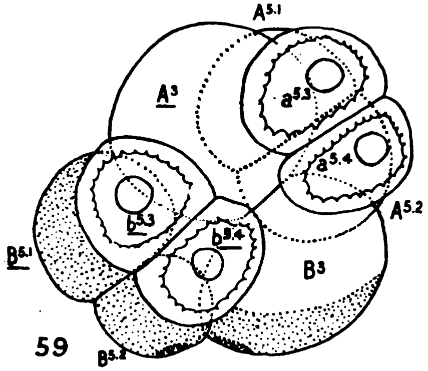
Fig. 60. Left posterior quarter embryo of the 44-cell stage, posterior view.

Fig. 61. Right anterior quarter embryo of the 76-cell stage, dorsal view, showing the neural plate and chorda cells of the right side.

Fig. 62. Right posterior quarter embryo of about the 180-cell stage, dorsal view (*cf.* Figs. 7, 8); spurted in the 4-cell stage, fixed 2½ hours later, showing 6 muscle and 2 caudal endoderm cells.

Fig. 63. Left anterior quarter embryo, dorsal view; spurted in the 4-cell stage, fixed 5 hours later. An invagination of the ectoderm cells has the appearance of a gastrula, but is probably the invagination of the neural plate.

Fig. 64. Left anterior and right posterior (diagonal) quarter embryos, dorsal view; spurted in the 4-cell stage, fixed 5 hours later. Muscle cells are found only in the posterior quarter.



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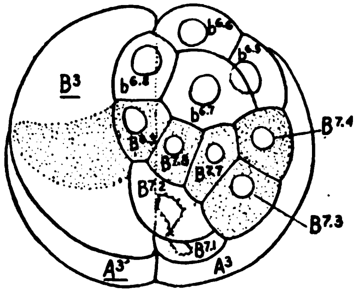

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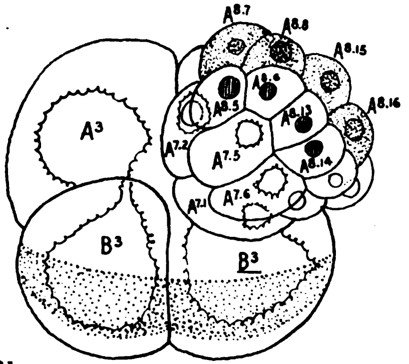

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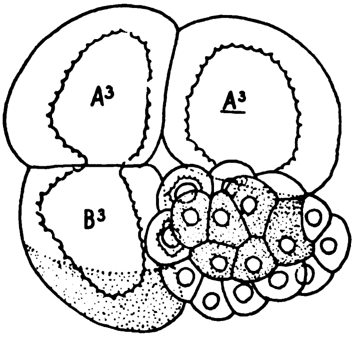

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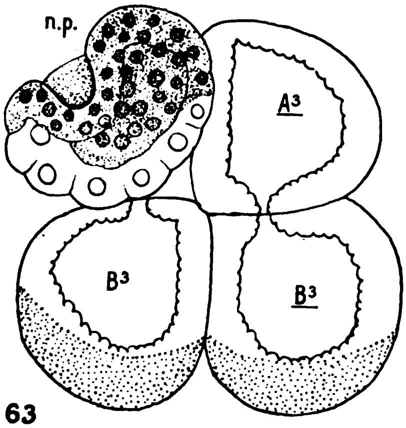

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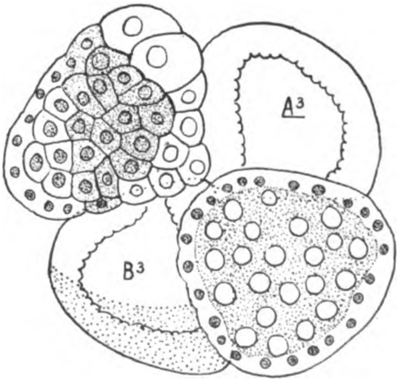

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QUARTER EMBRYOS; YOUNG TADPOLE TO METAMORPHOSIS STAGES. FIXED AND STAINED PREPARATIONS.

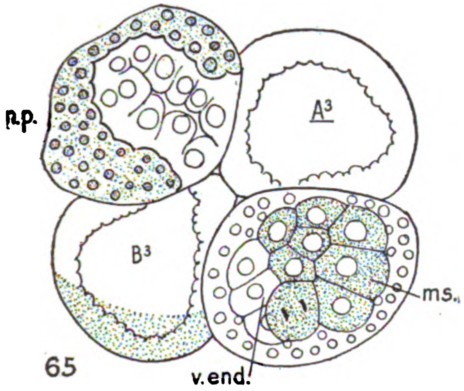
Fig. 65. Left anterior and right posterior (diagonal) quarter embryos, dorsal view; spurted in the 4-cell stage, fixed 5 hours later. The anterior quarter shows thickened ectoderm cells, probably neural plate, around the endoderm cells; in the posterior quarter are 8 muscle and 3 caudal endoderm cells.

Fig. 66. Left anterior and right posterior (diagonal) quarter embryos from the right anterior side, the dorsal pole being above; spurted in the 4-cell stage, fixed 22 hours later. In the posterior quarter the muscle and mesenchyme cells form a solid mass; in the anterior quarter the chorda cells project freely over the dorsal surface and the neural plate is partially infolded and contains three sense spots.

Fig. 67. Right anterior and left posterior (diagonal) quarter embryos, dorsal view; spurted in 4-cell stage, fixed 22 hours later.

Fig. 68. Left anterior and right posterior (diagonal) quarter embryos, dorsal view; spurted in 4-cell stage, fixed 22 hours later. In this and the preceding figure the chorda cells (Ch.), neural plate (n. p.) and sense spots are found only in the anterior quarters; the muscle, mesenchyme and caudal endoderm cells, only in the posterior quarters.

Figs. 69, 70. Right anterior quarter embryos, dorsal side above; spurted in 4-cell stage, fixed 12 hours later. These embryos show free chorda cells, neural plate and sense spots, but not a trace of muscle cells.



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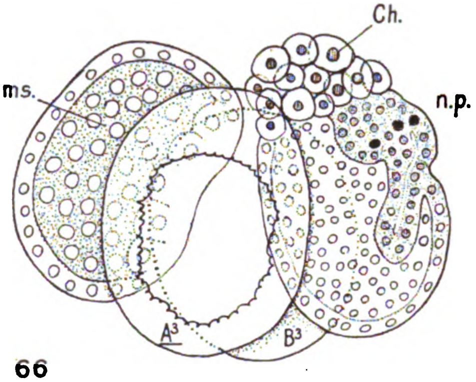

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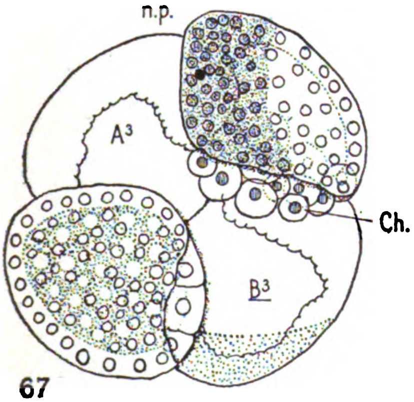

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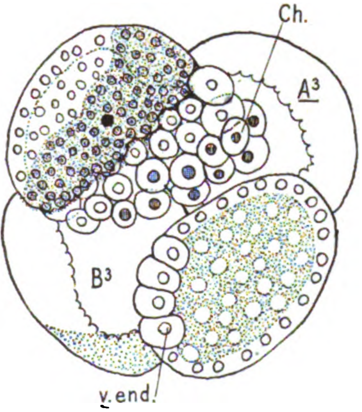

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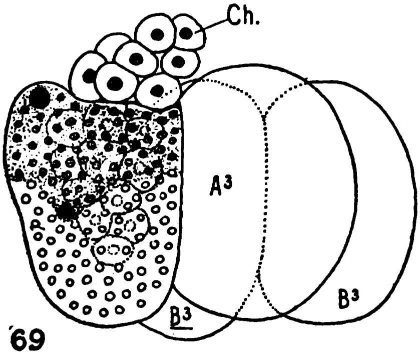

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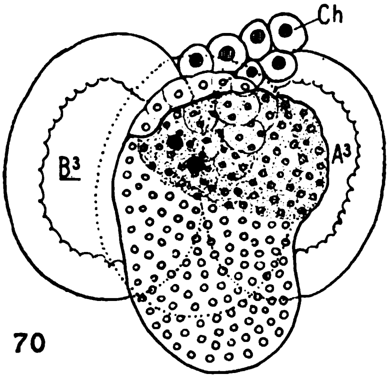

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derm cells remain on the rounded surface of the embryo. I have not observed in detail the process of gastrulation in any of these quarter embryos, but it is evident that there is no considerable gastrula cavity and that the endoderm cells are chiefly overgrown by the ectoderm, as shown in Fig. 65. Ultimately the endoderm and mesoderm are largely overgrown, though in this case, as in the half embryos, the ectoderm does not entirely inclose the embryo on the side next to the injured cells and through the opening thus left some of the endoderm cells may protrude.

Although the localization of oöplasmic substances and of organ bases is usually the same as in the quarter of an entire embryo, in some cases there are dislocations of these substances and bases which are probably due to injury of the surviving quarter. Thus in the left anterior quarter, shown in Fig. 64, large endoderm cells lie at the surface next to the first cleavage plane; in the same quadrant of another egg shown in Fig. 65 the neural plate cells lie at the periphery of the quadrant and chiefly on the left side, instead of along the median plane as in normal embryos. I have seen many other instances of such dislocations but they are all of such nature that they can be interpreted as due to slight injury to the surviving blastomeres. In not a single instance are parts derived from a blastomere which would normally have come from another cell.

The anterior quarter embryos are always recognizable by the presence of the neural plate and, in later stages, of the sense spots. The neural plate usually remains at the surface and is not infolded, but in some cases it is invaginated through at least a portion of its area, though a sense vesicle is not formed. (Figs. 63, 66.) In all later stages one or more sense spots appear in the plate. (Figs. 66–70.) The neural plate always lies along the dorsal side of the embryo, though it may be shifted more or less from the median plane. (Figs. 65–70.) The chorda cells are found exclusively in the anterior quadrants and in later stages they protrude to the exterior along the injured side where they are found as scattered cells in the perivitelline space. (Figs. 66–70.) In no case, save one, have I seen any indication that these cells form a rod-shaped notochord, and this case (Fig. 72) was that of a living embryo in which it is possible that the notochord-like structure was really composed of gastral endoderm and hence not a true notochord at all. It is evident that the chorda cells are unable to give rise to a notochord when once they have escaped and have become free, a certain amount of compression being necessary to bring about the characteristic interdigitation which leads to the formation of a rod-shaped notochord.[1]

The posterior quadrants can be distinguished in all eggs at all stages by the presence of the yellow crescent substance or cells. In early stages, as I have shown, these crescent cells are normal in position and character; in later stages the yellow cells fill the whole interior of the embryo. When once these cells have been inclosed by the ectoderm I have been unable to recognize any constancy in their position and arrangement. As in the posterior half embryos, a tail is never formed in these posterior quarter embryos and the muscle cells are never elongated, both these features evidently depending upon the presence of a notochord. The caudal endoderm cells are found in most, if not all, of these posterior quarter embryos as a single row of yolk laden cells which lie along the first cleavage plane (Figs. 65–68), the position which they normally occupy.

These quarter embryos show in the most unmistakable manner that the development is strictly partial, and that an individual blastomere never gives rise to parts which it would not produce in the entire embryo. Among the hundreds of quarter embryos which I have studied both in the living condition and as stained and mounted preparations I have never seen a single one which even remotely resembled a normal larva.

6. *Eighth or Sixteenth Embryos (Figs. 71–76).*

When eggs are spurted or shaken in the 8-cell and 16-cell stages a great variety of abnormal forms are produced, a few of which are shown in Figs. 71 and 73–76. Without exception, however, the same principles apply here as in the case of half and quarter embryos, viz: a given blastomere or group of blastomeres produces only those parts of an embryo or larva which would develop from it under normal conditions. Fig. 71 represents an embryo derived from the dorsal anterior eighth of an egg (the cell $A^{4.1}$) 14 hours after the injury. Normally this eighth gives rise to neural plate, chorda, gastral endoderm, and a small amount of

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[1] Chabry, however, figures (his Fig. 18) a partial embryo with a rod-shaped notochord lying outside the embryo in the perivitelline space.

PARTIAL EMBRYOS FROM ISOLATED BLASTOMERES OF 8-CELL OR 16-CELL STAGES. DRAWN FROM LIVING SPECIMENS.

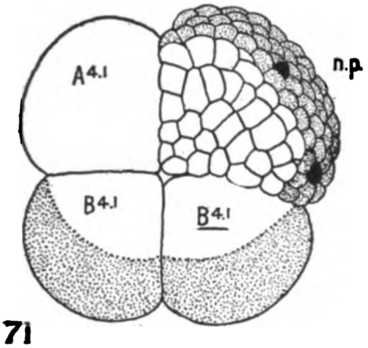
Fig. 71. Right anterior dorsal eighth embryo, 14 hours after injury, showing endoderm, chorda, and neural plate cells with sense spots.

Fig. 72. Right anterior quarter embryo, 14 hours after injury, showing chorda, neural plate, and sense spots.

Fig. 73. Posterior ventral quarter embryo derived from the cells $\mathrm{b}^{4.2}$, $b^{4.2}$ and containing no endoderm and only a small amount of yellow protoplasm which was derived from the perinuclear plasm of the cells $\mathrm{b}^{4.3}$, Fig. 13.

Fig. 74–76. Three views of a partial embryo derived from 7 cells of the 20-cell stage, viz: 2 ($\mathrm{B}^{5.2}$), 2 ($\mathrm{b}^{5.4}$), 1 ($\mathrm{a}^{5.4}$), 2 ($\mathrm{a}^{5.3}$). (*cf.* Figs. 3 and 4.) The embryo consists of an outer layer of clear ectoderm and of a mass of yellow mesenchyme cells derived from the cells $\mathrm{B}^{5.2}$, but it is wholly without endoderm.

Fig. 74, Ventral view; Fig. 75, Posterior; Fig. 76, Postero-dorsal.



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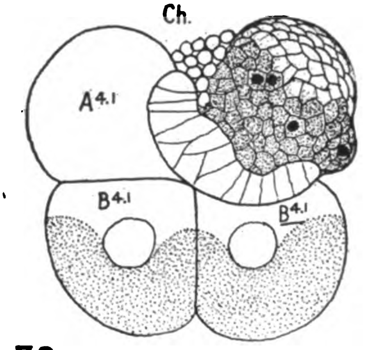

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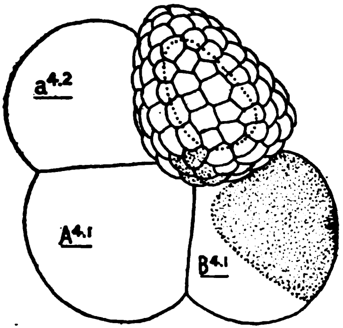

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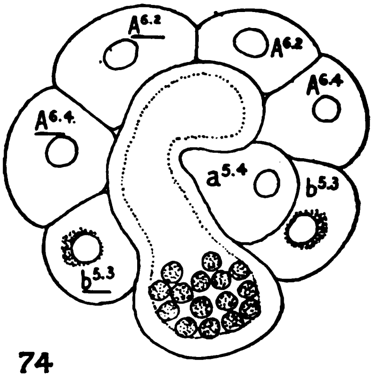

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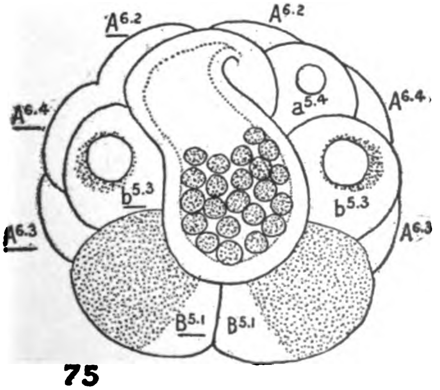

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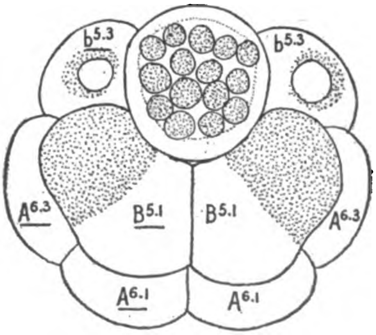

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mesenchyme derived from the cell $\mathrm{A}^{7.8}$. In the embryo shown in Fig. 71 the neural plate cells are clearly shown around the periphery of the figure and two of the cells contain sense spots. The chorda, endoderm, and mesenchyme cells are shown internal to the neural plate, but I am unable to distinguish in this embryo between these three kinds of cells; they are all more or less yolk-laden as in the normal egg. Owing probably to the fact that no ventral ectoderm cells are present the neural plate is not pushed up onto the dorsal face and there are no evidences of gastrulation, although normal embryos of a corresponding age have already reached the full larval development. That this failure to gastrulate is not due to the slower development of the egg fragments as compared with the entire egg is shown by the degree of histological differentiation of the neural plate and sense spots, the latter appearing normally only in the fully formed larvæ.

Fig. 72 is a quarter embryo of the same age as the preceding, derived from the cells $A^{4.1}$, $\mathrm{a}^{4.2}$ of the right anterior quadrant. The ventral ectoderm cells have here pushed the neural plate cells up onto the dorsal face of the embryo, while the chorda cells (?) lie along the median and transverse furrows. Four sense spots are present in the neural plate.

Fig. 73 is also a quarter embryo of the same age as the preceding, derived from the two posterior ventral cells $\mathrm{b}^{4.2}$, $b^{4.2}$. This embryo consists entirely of ectoderm which is arranged in a single layer of cells around a central cavity, the blastocoel. There has been no gastrulation and the embryo contains neither endoderm nor mesoderm. A few of the ectoderm cells next to the cell $A^{4.1}$ contain yellow pigment, exactly as in the normal embryo.

Figs. 74 to 76 are three views of one embryo, about 20 hours after the egg was spurted in the 20-cell stage. By the spurting all the cells were killed except seven from which this embryo has developed, viz: a pair of mesenchyme cells $\mathrm{B}^{5.2}$, and five ectoderm cells, $\mathrm{b}^{5.4}$, $b^{5.4}$, $\mathrm{a}^{5.3}$, $a^{5.3}$ and $a^{5.4}$. (See Fig. 3.) This embryo consists entirely of an outer layer of clear ectoderm cells, inclosing at its posterior end a mass of small mesenchyme cells; it contains no endoderm. It is an interesting fact that the mesenchyme cells are here inclosed by the ectoderm, showing that some process in the nature of gastrulation must have taken place.

A great many other partial embryos, produced from one or more blastomeres of the 8, 16 or 32-cell stages, have been studied but they all illustrate the principle that a blastomere never gives rise to any other structures than those which it would produce in a normal embryo.

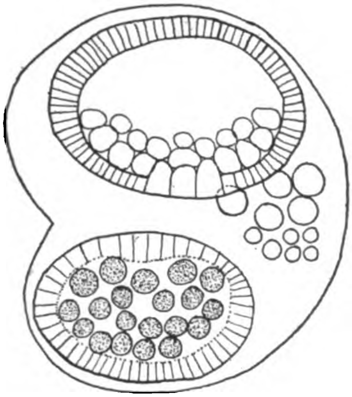
7. *Anterior and Posterior Half Gastrulæ* (*Figs. 77–82*).

In a recent publication Driesch ('03) has maintained that an alteration in the capacity for regulation occurs in the ascidian development between the early and the late gastrula stages. When the open cup-shaped gastrulæ of Phallusia were cut in two transversely into anterior and posterior halves, each of these halves developed into "einer vollständigen kleinen Appendicularie, welcher Organe niederer Bedeutung (Otolith, Augenfleck) eventuell fehlten." However, when the elongated gastrulæ were cut in two transversely a head developed from one piece and a tail from the other, "so deutlich und sharf begrenzt und ausgebildet, als habe man eine fertige Appendicularie scharf durchschnitten."

Considering the results which I have obtained on the development of the two anterior or two posterior cells of the 4-cell stage of Cynthia the conclusions of Driesch seemed most remarkable and I therefore undertook to repeat his experiments upon Cynthia. Gastrulæ of the stage shown in Fig. 8 were cut in two with a sharp knife made from a needle, under a Zeiss binocular dissecting microscope. With the power used the individual cells of the yellow crescent could be plainly seen and it was always easy to determine the exact boundary between the anterior and posterior halves. In every instance the section was made as close as possible to this boundary (second cleavage plane) and so as to leave all of the yellow cells in one of the pieces. Owing to the presence of the chorion the experiment was not an easy one to perform, since the chorion would frequently slip under the knife, or the egg move within the chorion. Nevertheless in one day I succeeded in cutting in two about thirty of these early gastrulæ; ten of these lived for twenty hours or longer after the operation, the others were too badly crushed to survive. Four of these which survived the operation are shown in Figs. 77–82, the drawings having been made from nineteen to twenty hours after the operation. Every one of these ten surviving embryos was a partial one and, although I was unable to determine their structure with the same amount of detail as in the case of stained and mounted preparations, it was

ANTERIOR AND POSTERIOR HALF GASTRULAE.

Figs. 77–82. Partial embryos derived from gastrulæ of the stage shown in Fig. 8, which were cut in two transversely so as to leave the whole of the yellow crescent in one half. The chorion is shown as a line around the embryos. Figs. 77, 78. Dorsal and ventral views respectively of one and the same embryo, drawn 19 hours after the operation. A mass of cellular débris lies between the two half embryos; the endoderm cells are chiefly contained in the anterior half, the mesenchyme and muscle cells are entirely confined to the posterior half. Neither half at all resembles a normal embryo or larva. Figs. 79, 80. Ventral and postero-dorsal views of another embryo, 19½ hours after the operation. The crescent of yellow cells is entirely confined to the posterior half and neither half resembles a normal larva. Fig. 81. Anterior and posterior half embryos 20 hours after the operation. Fig. 82. Posterior half embryo from the postero-dorsal side, 20 hours after the operation. The anterior half is degenerating and is shown only in dotted outline; the posterior half contains all of the yellow cells and practically no endoderm. At the stages represented by all these figures the normal embryos have already undergone their metamorphoses.



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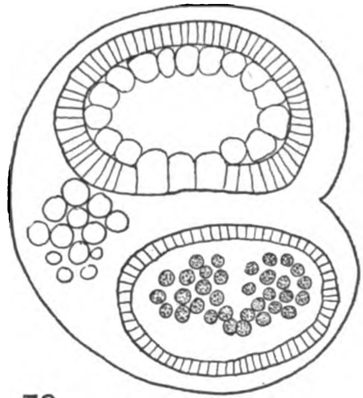

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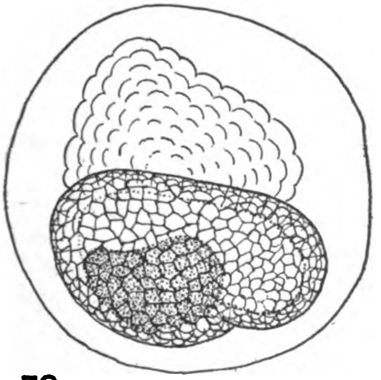

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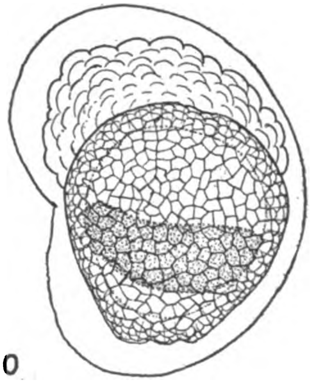

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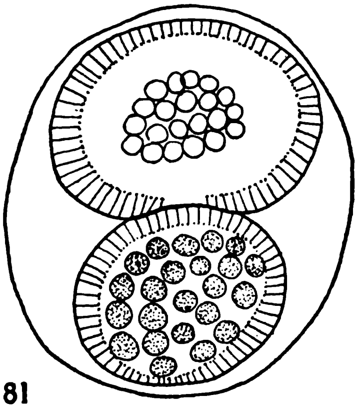

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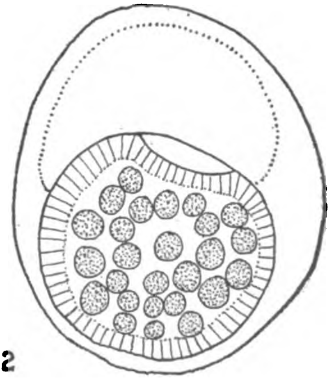

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quite evident that not one of them resembled in any respect whatever a normal larva. In some cases both halves survived, as shown in Figs. 77–81, in other cases one half only survived. In all cases the surviving halves became rounded in form after the operation, the more seriously injured cells being crowded out of the embryo and forming a cellular mass of débris within the chorion. In every instance the surviving halves remained within the chorion, which was sometimes infolded as shown in Figs. 77–80. Each half was surrounded by a layer of clear ectoderm cells; the yellow cells were always found exclusively in the posterior half, the gray endoderm cells largely in the anterior half. Nothing resembling a notochord or neural tube ever developed in either half and no structure resembling a tail was ever formed. In fact these half embryos produced by cutting the early gastrulæ in two were altogether like the anterior and posterior half embryos which I have already described. (*cf.* Figs. 77–82 and Figs. 47–58.)

These results were so definite and conclusive that I did not continue the experiments and I regret now that I did not also cut gastrulæ in two along the median plane, though there is no reason to doubt that the results would be the same as in cases where one of the first two blastomeres is killed.

Comparing these results with those of Driesch, only one of two explanations is possible. Either Phallusia must differ most fundamentally from Cynthia, or Driesch must have mistaken the median for the transverse plane in these cup-shaped gastrulæ. That the former possibility is not probable is evidenced by the fact that the cell-lineage of all ascidians so far studied is essentially the same; furthermore my results as to the development of anterior and posterior halves of the egg of Cynthia are confirmed by my experiments on Molgula, as well as by Chabry's experiments on Ascidia aspersa. There is every reason to believe that what is true of these three genera is also true of Phallusia. On the other hand there are certain evidences that Driesch may have mistaken the transverse plane for the median; on p. 56 he says, "Aber auch an der Bechergastrula kann man die künftige Mediane und also auch die Hauptrichtungen senkrecht zu ihr unterschieden: es verlaufen nämlich die Zelltheilungsgrenzen des Ektoderms dieser Objecte so, dass sie gerade in der Medianen eine über die ganze Oberfläche fortgesetzte, nur sehr wenig gebrochene Einheitslinie bilden (S. z. B. Castle, Fig. 62, 71) welche ohne Weiteres schon bei schwacher Vergrösserung kenntlich ist; schneidet man also in der Mitte und senkrecht zu dieser Linie, so zerlegt man auch die Bechergastrula in 'vorn' und 'hinten.'"

It is true that the median plane is marked out by a nearly straight line, though Castle's figures to which Driesch refers show this line between endoderm and not between ectoderm cells, but any one who has studied these embryos knows how difficult it is to determine the median plane in this way, especially in living material. Even in stained and mounted preparations it would not be a sure guide, much less could it be relied upon in the study of living gastrulæ. Whether the median plane appears as a straight line or not depends entirely upon whether that plane lies directly in the line of vision, and conversely some of the transverse planes of cleavage may appear as straight lines if they lie in the line of sight. Thus Fig. 7 shows several transverse rows of ectoderm cells which in the hinder part of the embryo are curved back in the middle and forward at the sides, but if the embryo were rotated forward so that the polar body were brought to the highest point these transverse rows would appear nearly straight.

I am convinced therefore that the half gastrulæ from which Driesch obtained apparently normal larvæ were right or left halves and not anterior and posterior ones as he supposed. Whether these larvæ were really normal, *i. e.*, whether they had the organs of both the right and left sides, cannot be determined from Driesch's figures or descriptions, since he seems to have considered that the only evidence required to show that a larva is complete is that it should have a head and a tail.

The fact that Driesch always obtained partial larvæ from the anterior and posterior halves of an elongated gastrula, where the chief axis is unmistakable, requires no comment.

IV. OTHER EXPERIMENTAL WORK ON THE ASCIDIAN EGG.

Chabry's ('87) contribution on the normal and teratological embryology of ascidians contains not only the most careful and complete experimental work which has ever been done on the ascidian egg but it is at the same time such an excellent analytical treatment of the normal development that it deserves to rank as an embryological classic. The experimental part of his paper was based upon an unusual knowledge of the normal and pathological development of this species and it was carried out with a delicacy and precision of method which has never been surpassed. Add to this the fact that the work was undertaken with clear insight into the principal problems involved and at a time when almost no other work of this sort had ever been done[1] and its right to rank as one of the great works in experimental embryology seems assured. Considering these facts it is surprising that this work should have received so little attention and that it should have been so widely misunderstood or discredited.

Chabry's extensive experiments deal with right and left half embryos, anterior and posterior two-quarter embryos, and various forms of three-quarter, one-quarter, and two-quarter diagonal embryos, and in all of these I find that my results are in the main in accord with his. The points in which my work is more detailed than his concern the presence and distribution of the various oöplasmic substances and the more accurate study of some of the later stages, made possible by the use of fixed and stained material. That the substance of the mesodermal crescent was seen by Chabry as early as the 32-cell stage is evident from his description of the mesoderm cells, which in Ascidia aspersa are greenish ("verdâtre") in color and which he recognized when only three were present on each side. Neither Driesch nor Crampton speak of having observed any of these oöplasmic substances and neither of them studied the later stages by means of fixed and stained material.

1. *Cleavage.*

Chabry showed that in rhythm of cleavage and in the size and character of the daughter cells the isolated blastomeres of Ascidia behave as if they were still part of the normal egg, while he described in great detail the changes which take place in the facets between cells. Crampton's conclusions are very similar; he found that "an isolated blastomere of the Molgula egg segments as if still forming a corresponding part of an entire embryo. The *cleavage phenomena* are strictly *partial*, as regards the origin of cells, the inclination of cleavage planes, and especially in respect to the rhythm of segmentation." Driesch, on the other hand, found in Phallusia that there was no fixed relation between the cleavage planes of the surviving half and the dead blastomere; that after the third cleavage the cells occupy very different positions from the normal (Tetraeder, Halbtetraeder); that divisions may be equal or unequal at the fourth cleavage, and finally that the cleavage could not be regarded as partial ("halb") nor entire ("ganz") but "regellos-solid." The evidence which Driesch brings in support of this conclusion is of little value since it is plain that he was unable to orient these cleavage forms and did not know from what part of the original egg they came nor from what pole they were viewed. My observations on the cleavage of isolated uninjured blastomeres of the egg of Cynthia confirm and extend the conclusions of Chabry and Crampton that the cleavage of such blastomeres is unaltered save for slight changes in the direction of some of the divisions; they are opposed to the conclusions of Driesch that the cleavage of such blastomeres is inconstant and irregular.

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[1] See Roux, Ges. Abhand II, p. 958.

2. *Gastrula.*

Chabry figures four gastrulæ from isolated blastomeres, viz: his Figs. 108, 114, 129 and 130. O. Hertwig, who copies Fig. 129 in his book, "Die Zelle" ('98), says that it is a normal typical gastrula. Similarly Korschelt and Heider, who also copy this figure in their text-book ('02), affirm that it is a normal small gastrula. However, these authors bring no particle of evidence to the support of this bare assertion; Chabry himself nowhere says that any of the gastrulæ figured by him are normal and the figures themselves do not show that such is the case. On the other hand I can positively affirm that a normal entire gastrula is never formed from an isolated blastomere of the egg of Cynthia. In the absence of any evidence in favor of Hertwig's and Korschelt and Heider's interpretation and in the face of this positive evidence against it I think it may safely be assumed that Chabry's figures are not those of normal typical gastrulæ. Crampton expressly says that he did not carefully observe the process of gastrulation in the embryos derived from isolated blastomeres of the Molgula egg, but Driesch says that the process of gastrulation may be easily observed in Phallusia, that a typical ascidian gastrula is formed and that the closure of the blastopore takes place in the normal manner. "Alles sind verkleinerte Aehnlichkeitsbilder der Processe an normalen Eiern, welche stets vergleichen wurden."

However, it is quite evident from the observations of Van Beneden and Julin, Chabry, Castle and many others that something more than a mere invagination is necessary to constitute a normal gastrula. The ascidian gastrula is bilaterally symmetrical and its anterior and posterior portions are very unlike; furthermore all the principal organs of the larva are here represented by cells of peculiar structure and localization. In order to determine whether a gastrula is normal or not all of these features have to be considered, and this Driesch has not done.

3. *Larva.*

It is somewhat surprising that doubt should have been expressed as to whether Chabry obtained half embryos or whole embryos of half size from one-half of the ascidian egg. He again and again declares that lesion of a single cell up to the 16-cell and probably up to the 32-cell stage always causes a "hemiterie," or monster. (Chabry, pp. 246, 249, 250, 257, 258, 261, etc.) He even enters into a calculation of the number of kinds of monsters which may be produced by injuries to the cleavage cells. He says that if at the 8-cell stage each cell is capable of four different kinds of modification (certainly less than the reality), the number of modalities of this stage is 4^8 (= 65536) of which only one is normal. In this way there arises that "admirable and infinite variety of monsters" to which he repeatedly refers. He says expressly, p. 289, "De là on tire aisément la conclusion (que je ne crois valable que pour l'Ascidie et les animaux, dont les blastomères sont différenciés de bonne heure), que chaque blastomère contient en puissance certaines parties dont sa mort entraîne la perte irrémédiable et que les différentes parties de l'animal sont préformées dans les différentes parties de l'oeuf." Again on p. 299 he says, "On ne saurait donc conclure avec sécurité de l'oeuf d'Ascidie à celui des autres animaux, mais, en ce qui concerne celui-ci, il est exact de dire qu'il se comporte comme s'il contenait en puissance un seul adulte déterminé et que chaque partie de l'oeuf contînt une partie de cet adulte." This same conclusion is repeated again and again so that as Barfurth ('93) and Driesch ('95) have said there can be no question as to what Chabry believed that his observations and experiments proved.

The statements of Driesch and Crampton are even more positive and explicit that whole larvæ are formed from any one or more of the first four blastomeres. Driesch (p. 405 in summarizing his results uses, in part, the very words of his conclusions regarding the value of the cleavage cells in the echinoderm egg: "Aus isolirt überlebenden Blastomeren des Ascidieneies entwickelt sich nicht ein halber (resp. viertel, drei viertel) rechter oder linker (resp. vorderer oder hinterer) Embryo, sondern stets ein ganzer von halber Grosse, dem allerdings (meist) gewisse Organe von minderen Bedeutung (Otolith, ein Haftorgan) fehlen." Crampton neither figures nor describes the larvæ obtained from isolated blastomeres of Molgula, but he says, p. 55, "Enough of the later development has been ascertained to prove that a larva arises which resembles the normal larva, except as regards its smaller size and certain minor defects. My results, therefore, are entirely confirmatory of those of Driesch upon Phallusia."

Chabry first discovered that larvæ from one of the first two blastomeres were superficially like normal larvæ in that they had head and tail, notochord, neural plate and sense spots, but he showed that they also lacked the organs distinctive of the missing side, viz: one papilla, one or more sense spots and one atrial invagination. It is surprising therefore that neither Driesch nor Crampton undertook to prove that the larvæ obtained by them from one of the first two blastomeres were really complete. One looks in vain in their papers for any evidence that the organs characteristic of that side which would have developed from the dead half (muscles, mesenchyme, papilla, atrial invagination) are present in the surviving half.

Chabry further showed that the type of embryo derived from the anterior or posterior two-quarters of the egg was very unlike that derived from the right or left two-quarters, while the one-quarter embryos were still more unlike the normal; in each of these cases he found that the development was strictly partial, only those parts arising from a blastomere which would develop from it in the normal embryo. In the face of these conclusions of Chabry's neither Driesch nor Crampton advance any evidence in favor of their claim that the anterior and posterior quadrants of the egg as well as the right or left may give rise to a larva. Chabry's figures and descriptions show plainly what my work proves that nothing even remotely resembling a normal larva is ever produced from any portion of an egg which does not include the whole of the right or left half. In my opinion Driesch and Crampton have not studied nor taken any account of anterior or posterior half embryos, but only of right or left ones. The question whether these embryos were actually complete will be considered when we come to deal with the various larval organs.

Both Driesch and Crampton make the claim that single blastomeres of the 4-cell stage of the ascidian egg may give rise to entire larvæ. This is a crucial test of their views, for while it is possible and I believe practically certain that all their "complete larvæ of half size" were derived from the right or left halves of the egg and so included portions of all the various oöplasmic substances, this explanation could not apply to their quarter embryos. Driesch figures a larva with all the principal organs (his Fig. 16), which he says is derived from one of the first four blastomeres. However, in size it is as large as any of the half larvæ which he figures, and I have no doubt that it is such.

Crampton figures correctly the early cleavages of one of the anterior quadrants and he gives two figures of quarter larvæ, probably of an advanced stage; these figures, however, show no structure whatever save that there is an outer layer around the embryo. There is absolutely no evidence that these embryos are complete. Crampton calls attention to the fact that the long axes of these quarter embryos "are approximately parallel to the principal dorso-ventral axis of the original egg," a fact which I also can confirm. (See my Figs. 66, 69, 70.) He does not, however, determine the fact, which he apparently assumes, that the long axes of these quarter embryos correspond to the long axis of a normal embryo. This is actually not true, as I have shown; the long axes of the quarter embryos are not antero-posterior in direction but dorso-ventral and there has not therefore been any shifting of the axes nor of the oöplasmic substances of these quarter embryos.

Whether a larva derived from the right or left half of the egg is complete or not can be determined only by a study of the various systems of larval organs. It is evident that parts of all organs which are normally formed along the median plane (first cleavage plane) would appear in an embryo derived from one of the first two cleavage cells, even if the development were strictly partial; the really decisive test as to whether such an embryo is complete or not must be found in the study of those organs which do not lie along the median plane.

a. Neural Plate and Sense Organs.

Chabry says that he never saw a partial embryo in which the neural plate had invaginated; on the contrary the nervous system always remains spread out in the form of a layer or plate; this plate occupies the face of the embryo which is morphologically median in position (its normal location), while the sense spots consist of pigmented cells which are superficial in position and which lie near the base of the tail. This agrees very closely with my observations, though I have frequently seen the neural plate invaginate by an irregular process. The eye is said by Chabry to be formed on the right side normally, but the fact that it may appear in the left half embryo leads him to conclude that its rudiment exists in the left half of the egg also. He thinks that the otolith comes only from the right posterior cell. I have not determined the exact cell origin of the sense organs in the normal larva, but in the partial larvæ they are formed only from the anterior quadrants and from either the right or left sides. I have not been able to distinguish between the eye and the otolith in the partial embryos of Cynthia.

Driesch says nothing of the neural plate nor of the manner in which the nervous system is formed in his small larvæ, though he mentions the fact that "the sense vesicle with the eye and otolith are not formed in the typically clear manner characteristic of the normal development." He found the eye spot almost always present, the otolith very seldom and he concludes that it makes no difference in the presence or absence of the sense organs whether the embryo has developed from certain cells of the 4-cell stage rather than from others. Since Driesch expressly states that he never raised a quarter embryo beyond the stage of his Fig. 16, at which stage the sense organs have not appeared, and since neither his figures nor descriptions give any evidence that he has distinguished anterior or posterior quadrants from right or left ones, it would be interesting to know how he could determine that sense organs might be formed from any quadrant of the egg—a result entirely contrary to my observations.

b. Notochord.

Chabry supposed that the notochord arose from both the anterior and posterior quadrants of the egg. Castle ('96) held that a single pair of cells of the posterior quadrants, $B^{8.6}$, "the posterior chorda fundament," were the only cells of the posterior quadrants which entered into the formation of the notochord. I am of the opinion that this cell is a mesenchyme and not a chorda cell (see Conklin, '05[1]), but even if it should be found to be a chorda cell it is only one cell of nine on each side of the mid line which give rise to that structure, while eight pairs of chorda cells come from the anterior quadrants. Certain it is that no trace of a notochord ever arises from the posterior cells when they are isolated, whereas chorda cells always arise from isolated anterior cells, though a notochord is rarely formed in such cases. Chabry describes (p. 294, Fig. 118) an anterior two-quarters embryo in which a naked chorda was seen in the perivitelline space outside the body of the embryo; such a case somewhat resembles the one shown in my Fig. 72. However, in every other instance which I have observed the chorda cells of an anterior embryo do not give rise to a notochord, but after escaping from the body of the embryo lie free in the perivitelline space as scattered cells. (Figs. 52, 66–70.)

But while a notochord is rarely or perhaps never formed in an anterior embryo and never in a posterior one, it is invariably found in a right or left one, and the figures of Chabry and Driesch as well as my own show that the process of formation is essentially the same as in a normal embryo. Chabry indeed believed that the notochord was primitively double and that half of it arose from each lateral half of the egg. He speaks of the fact that in Ascidia and Botryllus it is composed of a double row of cells and Crampton also refers to the fact that in the normal ascidian tadpole there are two rows of chorda cells, whereas Driesch has well said that in its fully formed condition the ascidian notochord is normally composed of a single row of cells. I find, as did Driesch, that the notochord of a lateral embryo is formed by interdigitation, just as in the normal embryo, but I also find, as opposed to Driesch that the notochord is never formed from any cells save the chorda cells which come from the posterior part of the gray crescent. Furthermore, my observations show, as did Chabry's, that the formation of a tail is dependent upon the development of a notochord.

c. Muscles and Mesenchyme.

Chabry paid no particular attention to the number and location of the muscle cells in his partial larvæ, though he frequently speaks of their presence as being proved by the twitchings of the tail; these movements are less energetic than in normal larvæ and, as a consequence, partial larvæ do not escape from the egg membranes. Driesch also found that partial larvæ rarely hatch, probably because of their weak muscular movements, but he, too, paid no attention to the number and position of the muscle cells. Owing to the brilliant color of these cells in Cynthia they are recognizable at all stages; in the partial larvæ they are found only along one side of the notochord, where they form the characteristic three rows of cells, whereas the muscle cells of the opposite side are entirely lacking. In the oldest larvæ a few of the muscle cells extend around the end of the notochord to the side on which they were lacking. I have not been able to determine whether the number of muscle cells is actually increased during this process or merely rearranged, but I believe that the whole process consists in the moving of certain cells over to the side on which they were lacking, without any increase in their number. This is part of that process of regulation which begins with the rounding up of the surviving blastomere after the other one has been killed. In fact, this very extension of the muscle cells around the end of the notochord begins in this rounding up of the surviving blastomere and in that slight change in the direction of division which causes the median cells of the yellow crescent to lie nearer the middle of the first cleavage plane than in the normal egg. (Fig. 15.)

Chabry found (p. 308, Fig. 132) only one atrial invagination and one organ of fixation (papilla) in right or left half embryos. Driesch did not determine the number of atrial invaginations but he does call attention to the fact that but one papilla is present in embryos from isolated blastomeres. I have not observed the formation of the atrial invaginations or of the papillæ in Cynthia; even in the normal larvæ they are inconspicuous at the time of the metamorphosis and I have not studied them before that period. However, the areas of trunk mesenchyme in which the atrial invaginations appear, are conspicuous areas of clear, slightly yellow, protoplasm in front of the muscle rows on each side of the tail; these areas may be recognized in the early cleavage stages and in no case are both these mesenchyme areas present in right or left half embryos. It is almost certain, therefore, that only one atrial invagination is formed in such embryos.

We find, therefore, that those parts of the larva which normally lie on the right side are missing in a left half embryo and those which normally lie on the left side are not found in a right half embryo, whereas unpaired organs which lie along the median plane are represented in both lateral half embryos. This is exactly what might be expected from a study of the organization of the egg since the substances, which give rise to median organs, are found along the median plane in both right and left blastomeres, whereas the materials which give rise to organs of the right side are found only in the right blastomere, those which give rise to organs of the left side, in corresponding positions in the left blastomere.

Neither Driesch nor Crampton attempt to show that a larva from the right half of an egg has the organs of the left side and this is the whole question at issue; if it does have these organs it is a complete embryo; if it lacks them it is a partial embryo, even if it does have a head and a tail. Chabry found that a larva from one of the first two blastomeres had a head and tail and median organs, but that it did not have the organs of the missing side and this conclusion I can entirely confirm.

All of the muscle substance (myoplasm) and most of the mesenchyme (chymoplasm) is localized in the posterior half of the egg, and corresponding to this distribution we find that an anterior half embryo entirely lacks muscles, though it may have a small amount of mesenchyme (that derived from the cell $A^{7.6}$), whereas a posterior half embryo contains a large number (probably the full normal number) of muscle cells and most of the mesenchyme.

V. REGULATION IN THE ASCIDIAN EGG AND EMBRYO.

It is well known from the work of Loeb ('92) and L. Schultze ('99) that the brain of Ciona will be regenerated when extirpated in the adult animal, and that the siphons will be restored when they are cut off. Driesch ('02) has also shown that Clavellina has extraordinary powers of regenerating almost all lost parts.

This power of regeneration in the adult is in striking contrast with its lack in the egg and embryo and requires some explanation.

It should not be overlooked that such injuries to the egg and embryo as have been described in the preceding pages are probably more extensive and far-reaching than any which are capable of being repaired in the adult. As Chabry says the destruction of one of the first two blastomeres is the same in its effect as the destruction of the right or left half of the body of an adult. The destruction of the anterior half of the egg is similar to the total loss of the nervous system and notochord of the larva; while the death of the posterior half corresponds to the destruction of the whole of the muscular system and most of the mesenchyme of the larva, since in each case the specific substance which alone gives rise to these organs is destroyed. Therefore these injuries are probably much more extensive than any which have been practiced on the adult animal.

Furthermore, I am of the opinion that the extremely rapid development of the ascidian egg and embryo may itself act as a check on regulation. In Cynthia and Ciona the fully formed larval stage is reached in about twelve hours after the fertilization of the egg, and these larvæ usually undergo metamorphosis into the adult form within the next twelve hours. In Molgula the development is even more rapid. It seems to me probable that the restoration of the parts of the missing right or left half of a larva might be fully accomplished if the larval life were longer. In a right or left half larva one day old the ectoderm cells have closed over the injured side, the notochord is complete, the neural plate has invaginated, although abnormally, and the muscle cells have begun to grow over from the uninjured to the injured side. There is here evidence of considerable regulative ability and it seems to me possible that, with more time before the metamorphosis, complete rows of muscle cells might be found on both sides of the tail and that the mesenchmye cells might grow over to the side on which they are lacking and an atrial invagination appear in them.

Inasmuch as the only form of regulation shown by the ascidian egg or embryo is this overgrowth of cells from the uninjured to the injured side, it is probable that no amount of time would ever suffice to produce an entire larva from the anterior or posterior half of an egg or from a quarter or any smaller portion. As a matter of fact there is not the slightest indication in an anterior half embryo of any attempt to restore the missing myoplasm or muscle cells, nor does a posterior half embryo show any tendency to form chorda-neuroplasm or neural plate or chorda cells. So far as observation and experiment show, each oöplasmic substance is capable of giving rise only to one particular kind of organ or tissue.

The question may be raised whether the presence of the injured blastomere within the chorion may not influence the development of the surviving cells and possibly prevent regeneration. In this and in all previous experimental work on the ascidian egg these injured cells have been left within the chorion in contact with the surviving cells and in this respect all work on these eggs has been done under similar conditions. Owing to the presence of the chorion it is practically impossible to remove the injured cells, and I am therefore unable to furnish an experimental test of the influence or lack of influence of these cells upon the surviving ones. However, there is sufficient evidence, I think, to show that it is not the presence of these cells which prevents regeneration. Contact with the injured cell might be expected to hinder or prevent the closing of the surviving half along the injured side, but it is just this form of regulation, and this only, which is manifested by these eggs. The presence of the injured cells can have nothing to do with the failure of the anterior half embryo to form a tail, or the posterior half embryo, a head; on the other hand, I have shown conclusively that the development of a tail is dependent upon the presence of a notochord, and the formation of a head upon the presence of the gastral endoderm and neural plate. The only possible influence of the injured cell upon the surviving one would be to limit the form-regulation; but as I have said this it does not do. It is inconceivable that the presence of the injured cell should prevent the myoplasm from giving rise to other organs than muscles, or the chorda-neuroplasm to other organs than chorda and neural plate.

These injured cells are rarely killed, but they remain transparent and entire, although quiescent; they do not decay and form a nidus for bacteria and I am convinced that their presence does not materially influence the development of the surviving half nor limit its powers of regulation.

VI. GENERAL CONCLUSIONS.

The conclusions which follow from these experiments are so obvious that they need but little emphasis here. Not only is the fact established that individual blastomeres give rise only to those parts of an embryo which they would produce under normal conditions, but the cause of this is clearly indicated. The development of the ascidian egg is a mosaic work because individual blastomeres are composed of different kinds of oöplasmic material; this mosaic work is not merely a cleavage mosaic but also a mosaic of germinal substances, several of which are recognizable before cleavage begins.

1. *Organ-Forming Substances.*

I have elsewhere shown that at least five distinct kinds of oöplasm are recognizable in the egg of Cynthia before the first cleavage and that all of these substances are localized in their final positions as early as the close of that cleavage. In these experiments I have not been able to isolate the different oöplasmic substances in the unsegmented egg, but after the second or third cleavages several of these substances may be isolated and in such cases each substance gives rise only to a definite kind of tissue or organ, and apparently it has no power to produce any other kind. The myoplasm produces muscle cells only; the chorda-neuroplasm, only chorda and neural plate cells; the chymoplasm, only mesenchyme; the endoplasm and ectoplasm only endoderm and ectoderm, respectively. Whenever an isolated blastomere lacks any of these substances, the embryo which develops from that blastomere lacks the corresponding organs. Accordingly the potencies of individual blastomeres are dependent upon the oöplasmic substances which they contain; the prospective value of any blastomere is not primarily a function of its position, but rather of its material substance.

The reason that the anterior quadrants of the egg never produce muscle cells is evidently due to the fact that they totally lack the yellow myoplasm; the fact that the posterior quadrants never produce a neural plate or chorda, is evidently due to the complete absence of the chorda-neuroplasm in these quadrants; the cells of the ventral (animal) pole produce only ectoderm, without a trace of endoderm or mesoderm,—evidently because these cells are composed almost entirely of clear ectoplasm.

Experiment confirms, therefore, what observation of the normal development plainly indicates that these strikingly different oöplasmic substances are not totipotent, but that as early as the close of the first cleavage and probably much earlier, they are differentiated for particular ends, and that if they develop at all they give rise to organs of a particular kind. These materials are, therefore, "organ-forming substances" and the areas of the egg in which they are localized are "organ-forming regions."

I need not here point out the similarity between this conclusion and the well-known theories of Sachs and His, nor the differences between my results and the commonly accepted view that the egg is composed of "simple undifferentiated protoplasm" or that "cleavage is a mere sundering of homogeneous materials capable of any fate," or that "the prospective value of a blastomere is a function of its position." Whatever may be true of other animals these things are certainly not true of ascidians.

While there are few, if any, other cases known in which the differentiations of the oöplasm are so striking or so numerous as in the egg of Cynthia there can be no doubt that organ-forming substances are present in the eggs of many animals. In particular the works of Fischel ('97, '98, '03) on the Ctenophore, of Boveri ('01) on Strongylocentrotus; of Wilson ('04) on Dentalium and Patella and of Conklin ('03) on Physa, Planorbis and Limnæa have shown that distinct kinds of protoplasm are present in these eggs which are destined in the course of development to give rise to particular germ layers or organs. In the light of these discoveries it can scarcely be doubted that the general cause of mosaic development is to be found in the presence in the egg or blastomeres of distinct kinds of protoplasm, or of organ-forming substances.

2. *Localization of Oöplasmic Substances.*

The three principal substances in the egg of Cynthia, viz: the clear, the yellow and the gray, are already present and localized in the oöcyte before it escapes from the ovary. The yellow (mesoplasm) forms a peripheral layer around the entire egg; the clear (ectoplasm) is the clear achromatic substance within the germinal vesicle; the gray (endoplasm) constitutes most of the remainder of the egg.[1] For the sake of brevity this earliest form of localization may be described as concentric or spherical, although the germinal vesicle does not lie exactly in the center of the egg but is slightly eccentric toward one pole.

During maturation and fertilization this concentric localization gives place to a polar or radial form. Immediately after the entrance of the spermatozoon into the egg the peripheral layer of yellow mesoplasm flows rapidly to the lower pole where it collects in the form of a cap; the clear ectoplasm which escapes from the germinal vesicle at first lies at the animal pole where it surrounds the maturation spindles but after the entrance of the spermatozoon it also flows to the lower pole where it collects into a layer or stratum just above the mesoplasm; the gray endoplasm after these movements occupies almost all of the upper half of the egg. The egg at this stage appears to be radially symmetrical, the three principal substances being arranged in strata at right angles to the egg axis.

Soon after the entrance of the spermatozoon this radial form of localization gives place to a bilateral one; the sperm nucleus and aster move up to the equator of the egg along one meridian which further development shows to be the median plane on the posterior side; the clear and yellow substances also move to the posterior pole along with the sperm nucleus and the yellow substance here forms a crescent around the posterior side of the egg, just below the equator. At this stage the egg is bilaterally symmetrical, there being but one plane which will divide equally all of the oöplasmic substances.

Finally during the first cleavage this early bilateral localization is changed into the definitive localization which is characteristic of all stages up to the late gastrula. The yellow crescent remains in the position which it occupied before the first cleavage and here gives rise to muscle and mesenchyme cells; the clear protoplasm comes to occupy most of the ventral hemisphere and gives rise to ectoderm; the gray substance occupies the dorsal hemisphere in front of the yellow crescent and its anterior portion becomes the gray crescent of chorda-neuroplasm, while its posterior portion is the deep gray endoplasm which gives rise to the gastral endoderm.

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[1] Although I have not been able to isolate these various oöplasmic substances before cleavage begins and, therefore, can bring no experimental evidence to prove that they are organ-forming substances at this early stage, it nevertheless seems probable that materials which are identical in color and texture with the organ-forming substances of later stages, to which they directly give rise, are also similar in potency. There is no apparent reason for believing that these strikingly different kinds of oöplasm of the ovarian egg are any less distinct or more nearly totipotent than during the cleavage stages.

The form of localization of these substances, therefore, undergoes marked changes during the fertilization and first cleavage; it is concentric in the oöcyte, polar or radial immediately after the entrance of the sperm, bilateral just before the first cleavage, and definitive at the close of the first cleavage.

I have elsewhere ('05[1]) shown reason for believing that even in the stage of radial localization in the egg of Cynthia there is probably some structural peculiarity of the egg which determines that the path of the sperm shall lie in one meridian rather than in another and therefore that the median plane of the embryo and its posterior pole are not determined by the chance movements of the sperm within the egg. Similarly the basis for polar or radial localization is present in the ovarian egg in the slight eccentricity of the germinal vesicle toward the animal pole, though the oöplasmic substances are largely localized in concentric form at this stage. I am unable to determine whether any structural basis for bilateral localization exists in the ovarian eggs of ascidians, but inasmuch as the localization invariably becomes bilateral at a later stage it seems necessary to suppose that there is some such intrinsic determinative factor.

In almost every group of animals the chief axis of the egg is already marked out in the oöcyte, the pole toward which the germinal vesicle is eccentric becoming later the animal pole of the egg and the ectodermal pole of the embryo. Despite this eccentricity of the germinal vesicle the localization of oöplasmic substances in the oöcyte of ctenophores, nemertines, echinoderms and ascidians is chiefly concentric, the polar localization of these substances first appearing during the maturation and fertilization. On the other hand Wilson ('04) has found a markedly polar localization of the oöplasm in the oöcyte of Dentalium; while it is probable that in the oöcytes of insects and cephalopods the localization is bilateral in form.

Boveri ('01) found that distortion of the egg of Strongylocentrotus after the formation of the equatorial zone produced no change in the polar stratification of the egg nor in the potencies of its different substances. Wilson ('03), Yatsu ('04) and Zeleny ('04) have discovered that fragments from any part of the egg of

Cerebratulus before maturation may give rise to entire larvæ; whereas this is not usually the case after maturation and fertilization, the potencies of the substances at the animal and vegetal poles being different. It is evident that during the concentric stage of localization section of an egg in any plane would leave samples of all the oöplasmic substances in each piece; in the stage of polar-radial localization any section of the egg parallel with the egg axis would leave samples of all the oöplasmic substances in each piece; in the bilateral stage, only the right or left halves would contain parts of all the substances. Probably one important reason why parts of eggs may give rise to whole embryos in some cases and not in others may be found in the fact that at the time of the experiment the form of localization may have been concentric in some cases, radial in others and bilateral in still others. (See Boveri, '01; Wilson, '04[1].)

3. *Cleavage and Localization.*

In previous publications ('05[1], '05[2]) I have pointed out the fact that localization precedes cleavage in the ascidian egg and that the localization pattern does not closely coincide with the cleavage pattern. On the other hand there is normally a constant relation between particular cleavage planes and the various oöplasmic substances. The first cleavage always lies in the median plane and bisects all the oöplasmic substances; the second is transverse to the median plane and separates the yellow crescent from the gray one; the third cleavage is at right angles to the two preceding ones and separates the clear ectoplasm of the ventral hemisphere from the different substances of the dorsal hemisphere. Probably in no other animal is the cleavage so constant and so perfectly bilateral as in the ascidians and yet even here the position and direction of the cleavage planes is less constant than the form of localization.

Among annelids and mollusks, as is well known, the cleavage is typically spiral and in many cases it is radially symmetrical. This radial symmetry of cleavage does not indicate, however, that the localization of oöplasmic substances is also radially symmetrical, for in some cases this localization is known to be bilateral and this is probably true in all cases. (See Conklin, '05[1], pp. 90–92.)

The relation of the cleavage planes to this bilateral organization is very different in cases of spiral and of bilateral cleavage, and consequently the results of killing any one or more of the first four blastomeres may vary in different cases; in general there is less likelihood of obtaining an entire embryo from an isolated blastomere of spiral cleavage than from one of the first two blastomeres in bilateral cleavage.

In other cases the cleavage planes bear no constant relation to the planes of localization. Thus in the frog's egg the first cleavage may lie in the median plane or at varying angles to this plane and Brachet ('04) has recently shown that the character of an embryo derived from one of the first two blastomeres depends entirely upon the relation between the first cleavage plane and the median plane of organization.

It is probable that the bilaterality of organization is no more perfect in ascidians than in annelids, mollusks or amphibians, but the bilaterality of cleavage is much more perfect. Accordingly, each of the first two blastomeres of the ascidian egg always contains half of every oöplasmic substance, in the frog's egg it may or may not contain half of these substances, in the annelid or mollusk it never does.

I agree therefore with Brachet ('04) and Wilson ('04^1, '04^2) that the varying results of experiments on the potencies of blastomeres are due in part to the varying relations of cleavage to localization, and in part also to the different types of localization (concentric, radial, bilateral) in different eggs.

4. *Determinate and Indeterminate Cleavage and Development.*

In a great many animals belonging to phyla as widely separate as Ctenophora, Polyclada, Nemertinea, Nematoda, Rotifera, Annelida, Mollusca, Arthropoda and Tunicata the cleavage of the egg is constant in form and differential in character and under normal conditions, definite cleavage cells always give rise to definite structures of the embryo or larva. For this type of cleavage I proposed several years ago ('97, '98) the designation "determinate." In a few animals the cleavage is known to be extremely irregular, as in Pennaria (Hargitt, '04), Renilla (Wilson, '84), and probably also in planarians (Hallez, '87; Stevens, '04), while in other cases it is unknown whether the cleavage is normally constant and differential or not (Echinoderms); in still other cases the planes of cleavage bear no constant relation to the planes of localization, as in the eggs of some of the vertebrates (frog, fish). For all such cleavages I proposed the name "indeterminate," but at the same time I was careful to state that this was "to be understood as applying only to the cleavage, for in its main features and results the development of all animals is determinate; that is, predictable. Even in cnidaria, echinoderms and vertebrates there appears successively a blastula, gastrula, larva, and adult of determinate form and character" ('98, p. 21).

But while the cleavage is indeterminate in some cases there is reason to suppose that there is a definite organization of the egg in all animals—in short that the organization of the individual is determinate at all stages from the egg to the adult. Even in such an egg as that of Pennaria it is certain that there must be determinative factors somewhere, if not in the cytoplasm then in the nucleus, which determine that the egg shall develop into a Pennaria rather than into some other animal; and it is further evident that these determinative factors must be present in the cytoplasm at a relatively early stage, if not at the very beginning of development.

In the echinoderm egg, which was at one time supposed to be homogeneous or isotropic, Boveri ('01) has shown that a polar-radial localization of at least three distinct morphogenetic substances takes place immediately after maturation, and in this case, as in the ascidians it is probable that there is an earlier concentric localization of these substances in the oöcyte. Since these three substances are localized in zones or strata, one above the other, around the chief axis of the egg, they are all present in each of the first four blastomeres of the egg, each of which may give rise to an entire embryo; but when they are isolated each is found to be strictly limited in its potentialities.

While therefore there are several groups of animals in which the cleavage is indeterminate there are few or none in which the oöplasm is isotropic; on the contrary in almost every phylum the eggs and blastomeres show differentiations and localizations of the oöplasm which are of morphogenetic value. "Everywhere," as Fischel ('03) has well said, "the fundamental principle of normal development is a mosaic work." But while Fischel supposes that "only the materials for the primitive organs of the embryo are preformed in the egg cell and that the material substratum for the differentiation of the special organs is probably first formed during the later stages," I find that in the ascidian egg all the principal organs of the larva are represented by distinct organ-forming substances which are localized in their definitive positions and proportions as early as the close of the first cleavage.

There is a world-wide difference between such results as these and those which were reached by Driesch and some of the earlier workers in this field. Wilson ('04) has recently expressed the opinion that "had the experimental analysis of cleavage been first undertaken in the case of such a determinate type as that of the gasteropod or annelid and had Roux not handicapped his theory with a purely speculative hypothesis of differentiation, which proved to be untenable, the whole discussion would have taken a different course; and I believe it would from the first have been recognized that the mosaic principle holds true in greater or less degree for every type of development, not excepting the most 'indeterminate' forms of cleavage." Considering the fact that such highly determinate forms as the ascidian and the ctenophore were studied in some of the earliest experiments on the potency of cleavage cells, I am of the opinion that the course which this discussion took was not primarily due to the fact that work began on relatively indeterminate forms. On the other hand I am convinced that the whole trend of opinion on the organization of the egg and on the potency of cleavage cells would have been different if those who did this work had been more familiar with the normal development of the forms studied, and in their zeal for the experimental method had not discarded the old and approved method of observation. It has taken such careful observers of normal processes as Boveri and Wilson to apply most successfully the experimental method to the problem of the organization of the egg, and the results of such work constitute a well-deserved tribute to the permanent value of the work of Roux.

SUMMARY.

I. *Normal Development.*

1. In the ovarian egg of Cynthia (Styela) partita there are three strikingly different kinds of oöplasm, viz: a superficial yellow layer, a central gray area, and a large transparent germinal vesicle. At this stage the localization of these substances is approximately concentric.

2. During maturation and fertilization the yellow substance flows rapidly to the vegetal pole where it forms a superficial layer or cap; the clear substance escapes from the germinal vesicle and flows toward the vegetal pole where it forms a stratum above the yellow cap; the gray substance occupies the animal half of the egg. The localization at this stage is polar-radial.

3. The sperm nucleus which lies in the center of the yellow cap moves to the posterior pole of the egg and the yellow and clear substances move with it. The yellow material here forms a crescent which lies with its center at the posterior pole and its arms extending forward on each side about halfway around the egg; the clear substance forms a band just above and internal to the crescent; the gray substance occupies the remainder of the egg. At this stage the localization is bilateral.

4. The first cleavage furrow appears in the plane of bilateral symmetry and divides each of the oöplasmic substances equally. At the close of this cleavage the clear substance occupies the animal (ventral) half of the egg; the gray substance lies at the middle of the vegetal (dorsal) pole while around the posterior border of the dorsal hemisphere is the yellow crescent and around its anterior border is a light gray crescent. This is the definitive localization of these substances, and in these positions the clear material gives rise to ectoderm, the gray to endoderm, the yellow crescent to muscles and mesenchyme, and the gray crescent to chorda and neural plate.

5. The second cleavage is transverse to the antero-posterior axis and separates the gray crescent from the yellow; the third cleavage separates the clear protoplasm of the ventral hemisphere from the various substances of the dorsal hemisphere.

II. *Experiments.*

6. Individual blastomeres were injured by spurting or shaking the eggs in the 2, 4, 8, or 16-cell stages. The surviving blastomeres were then studied both in the living condition and after being stained and mounted.

7. *Cleavage.* Isolated blastomeres always segment as if they still formed part of the whole, except that the direction of some of the cleavages is slightly altered so that the resulting cell mass is more nearly spherical than in the normal egg. These alterations

in the direction of cleavage and the consequent closing of the injured side are more apparent in isolated blastomeres of the 2-cell stage than in those of later stages.

8. *Gastrulation.* In right or left or anterior halves, gastrulation usually proceeds as if the fragment still formed part of the whole; even though the gastrula may be rounded in form the location of the different substances shows that it is strictly partial. Not infrequently isolated blastomeres give rise to exogastrulæ, which ultimately right themselves. In posterior halves and in quarter embryos, gastrulation does not at all resemble the normal process, either in methods or results.

9. *Right or Left Half Embryos.* A lateral half embryo is usually closed along the injured side; it has a head and a tail; a typical notochord, which is formed only from the chorda cells of the surviving side, and which is therefore composed of half the normal number of cells; an atypical neural plate and sense vesicle, formed only from the typical neural plate cells of the surviving side; a typical mesenchyme area in which the atrial invagination of one side is formed and three typical rows of muscle cells on one side of the notochord, but none along the injured side. In the latest stages to which these lateral embryos were reared (corresponding to the period of metamorphosis in normal larvæ) the muscle cells have begun to grow around the hinder end of the notochord to the side on which they were lacking; but in no case are the three rows of the normal embryo present on this side. Probably only one atrial invagination and one papilla are ever formed in these lateral embryos. These are therefore half embryos in which some cells have grown over from the uninjured to the injured side, but in which absolutely no change has taken place in the potency of the individual cells or of the different oöplasmic substances.

10. *Anterior Half Embryos.* Embryos derived from the two anterior quadrants of the egg have no trace of muscle cells nor of muscle substance; although the normal number of chorda cells are present they rarely if ever form a notochord but usually escape from the body of the embryo and lie scattered in the perivitelline space; the neural plate cells are present in normal number and position but the plate rarely, if ever, invaginates to form a sense vesicle; in late stages sense spots are formed from certain cells of the neural plate; cells of the gastral endoderm and general ecto-

derm are frequently present in their normal positions and numbers. A tail is never formed in these anterior embryos and they bear no resemblance whatever to a normal larva.

11. *Posterior Half Embryos.* Embryos derived from the two posterior quadrants have no trace of notochord or of chorda cells, neural plate, sense vesicle, sense spots, or gastral endoderm; they contain a mass of muscle and mesenchyme cells and a double row of caudal endoderm cells, as in the normal embryo. There is no indication of a tail or head, the embryo remaining rounded in form as long as it lives.

12. *Three-Quarter Embryos.* Embryos derived from three of the first four blastomeres are more nearly perfect than are half embryos, but they always show defects corresponding to the missing blastomere. If an anterior blastomere is killed, the neural plate and sense vesicle of the resulting larva are atypical and the notochord lacks the normal number of cells; if a posterior cell is killed, the muscle and mesenchyme cells are lacking along one side of the tail.

13. *One-Quarter Embryos; Two-Quarter Diagonal Embryos.* Embryos derived from any one quadrant or from two diagonal quadrants of the egg are always very defective. They never have a notochord, though if they come from anterior quadrants they may give rise to scattered chorda cells in the perivitelline space; there is never a sense vesicle, though if they are from an anterior quadrant a neural plate and sense spots are present. The posterior quadrants always contain muscle, mesenchyme and caudal endoderm cells, but never a trace of notochord, neural plate nor sense spots. The embryos are always rounded, there being no distinction of head and tail, and in no respect do they resemble normal larvæ.

14. *Eighth and Sixteenth Embryos.* When blastomeres are injured in the 8-cell or 16-cell stages a great variety of abnormal forms are produced. Ventral blastomeres give rise only to rounded masses of ectoderm cells in which there is no trace of endoderm or mesoderm; posterior dorsal cells give rise only to muscle, mesenchyme, and caudal endoderm; anterior dorsal cells to neural plate, chorda, and gastral endoderm.

15. *Anterior and Posterior Half Gastrulæ.* When cup-shaped gastrulæ of the stage shown in Fig. 8 are cut in two transversely so as to leave all of the yellow cells in one half and all of the chorda

and neural plate cells in the other a notochord, sense vesicle, or tail is never formed and nothing resembling a normal larva develops from either half. The anterior half never contains muscle cells; the posterior half contains many muscle and mesenchyme cells, but evidently no chorda or neural plate cells.

III. *Conclusions.*

16. My results confirm and extend those of Chabry, but they are fundamentally unlike those of Driesch; I agree with the work of Crampton as to the cleavage of isolated blastomeres but cannot agree with him that whole embryos or larvæ are ever formed from isolated blastomeres of the ascidian egg.

17. Regulation in the ascidian egg and embryo is limited to the closing of the embryo and the consequent extension of certain cells from the uninjured to the injured side; and also to the formation of a typical notochord and an atypical sense vesicle in right or left half embryos. One oöplasmic substance never gives rise to another nor does a given type of cell ever produce cells of another type or organs of a different kind than those which would arise from it in a normal embryo. The fact that the power of regulation is apparently greater in the adult ascidian than in the egg or embryo may be deceptive; the injury to the egg which wipes out completely certain oöplasmic substances may be really greater than any which may be repaired in the adult. Furthermore it is possible that the very rapid development of ascidians may act as a check on regulation.

18. These results prove that at least five of the substances which are present in the egg at the close of the first cleavage, viz: ectoplasm, endoplasm, myoplasm, chymoplasm, and chordaneuroplasm, are organ-forming substances. They develop, if they develop at all, into the organs which they would normally produce; and conversely, embryos which lack these substances, lack also the organs which would form from them. Although I have been unable to test the potencies of these substances before cleavage begins, there seems to be no reason for supposing that they are ever totipotent. Three of these substances are clearly distinguishable in the ovarian egg and I do not doubt that even at this stage they are differentiated for particular ends.

19. A possible explanation of the fact that all the fragments of an immature egg may give rise to entire larvæ, whereas the proportion which gives rise to larvæ steadily decreases after maturation and fertilization, may be found in the fact that before maturation the localization of oöplasmic substances is usually concentric, after maturation and fertilization, polar-radial; while just before or shortly after the first cleavage the localization may become bilateral. Also the fact that isolated blastomeres may give rise to whole embryos in some animals and to partial ones in others may be due to the varying relations of cleavage planes to localization planes. If at the close of the second cleavage the localization is still radially symmetrical, each of the first four blastomeres would probably be capable of giving rise to an entire larva; if the first cleavage invariably lies in the plane of bilateral symmetry, as in ascidians, each of the first two blastomeres might be capable of giving rise to an entire larva (though my work shows that this would not necessarily happen); if the cleavage planes do not coincide with the planes of localization, as in mollusks and annelids, isolated blastomeres would not give rise to entire larvæ. (See Wilson, '04[1], '04[2].)

20. The development of ascidians is a mosaic work because there are definitely localized organ-forming substances in the egg; in fact the mosaic is one of organ-forming substances rather than of cleavage cells. The study of ctenophores, nemertines, annelids, mollusks, ascidians and amphibians (the frog) shows that the same is probably true of all these forms and it suggests that the mosaic principle may apply to all animals. (*cf.* Fischel, Wilson.)

LITERATURE CITED.

Barfurth, D., '93.—Halbbildung oder Ganzbildung von halber Grosse? Anat. Anz., viii.

Beneden, van et Julin, '84.—La segmentation chez les ascidiens dans ses rapports avec l'organization de la larve. Arch. de Biol., v.

Boveri, Th., '01.—Ueber die Polaritat des Seeigeleies. Verh. Phys. Med. Ges., Würzburg, xxxiv.

'01.—Die Polaritat von Ovocyte, Ei und Larve des Strongylocentrotus lividus. Zool. Jahrb., xiv.

Brachet, A., '04.—Recherches expérimentales sur l'oeuf de Rana fusca. Arch. de Biol., xxi.

CASTLE, W. E., '96.—The Early Embryology of Ciona intestinalis, Flemming L. Bull. Mus. Comp. Zool., xxvii.

CHABRY, L., '87.—Contribution à l'embryologie normale et teratologique des Ascidies simples. Journ. Anat. et Physiol., xxiii.

CONKLIN, E. G., '97.—The Embryology of Crepidula. Jour. Morph., xiii.

'98.—Cleavage and Differentiation. Woods Hole Biol. Lect.

'03.—The Earliest Differentiations of the Egg. Science, xvii.

'05^1.—The Organization and Cell-Lineage of the Ascidian Egg. Jour. Acad. Nat. Sci., Philad., xiii.

'05^2.—Organ-Forming Substances in the Eggs of Ascidians. Biol. Bull., viii.

CRAMPTON, H. E., '97.—The Ascidian Half-Embryo. Ann. New York Aca. Sci., x.

DRIESCH, H., '95.—Von der Entwicklung einzelner Ascidienblastomeren. Arch. Entw. Mech., i.

'02.—Studien über das Regulationsvermögen der Organismen. 6 Die Restitutionen der Clavellina lepadiformis. Arch. Entw. Mech., xiv.

'03.—Ueber Anderung der Regulationsfahigkeiten im Verlauf der Entwicklung bei Ascidien. Arch. Entw. Mech., xvii.

FISCHEL, A., '97, '98.—Experimentelle Untersuchungen am Ctenophorenei. I and II Arch. Entw. Mech., vi, vii.

'03.—Entwicklung und Organ-Differenzirung. Arch. Entw. Mech., xv.

HALLEZ, P., '87.—Embryogénie des Dendrocoeles d'eau douce. Paris.

HARGITT, C. W., '04.—The Early Development of Pennaria tiarella. McCr. Arch. Entw. Mech., xviii.

HERTWIG, O., '92.—Urmund und Spina Bifida. Arch. Mik. Anat., xxxix.

'98.—Die Zelle, II. Jena.

KORSCHELT UND HEIDER, '02.—Lehrbuch der vergleichenden Entwicklungsgeschichte. Allgemeine Theil. Jena.

LOEB, J., '92.—Untersuchungen zur Physiologischen Morphologie der Thiere, vi and vii.

ROUX, W., '95.—Gesammelte Abhandlungen über Entwicklungsmechanik der Organismen. Bd. II, Leipzig.

'92.—Ueber das Entwicklungsmechanische Vermogen jeder der beiden ersten Furchungszellen des Eies. Verh. d. Anat. Ges. zu Wien.

'03.—Ueber die Ursachen der Bestimmung der Hauptrichtungen des Embryo im Froschei. Anat. Anz., xxiii.

Schultze, L. S., '99.—Die Regeneration des Ganglions von Ciona intestinalis L. und über das Verhaltnis der Regeneration und Knospung zur Keimblatterlehre. Jena. Zeit., xxxiii.

Stevens, N. M., '04.—On the Germ Cells and the Embryology of Planaria Simplicissima. Proc. Acad. Nat. Sci., Philad.

Weismann, A., '92.—Das Keimplasma. Jena.

Wilson, E. B., '84.—The Development of Renilla. Philos. Trans., clxxiv.

'03.—Experiments on Cleavage and Localization in the Nemertine Egg. Arch. Entw. Mech., xvi.

'04[1].—Experimental Studies on Germinal Localization, I and II. Jour. Exp. Zoöl., i.

'04[2].—Mosaic Development in the Annelid Egg. Science, xx.

Yatsu, N., '04.—Experiments on the Development of Egg Fragments in Cerebratulus. Biol. Bull., vi.

Zeleny, C., '04.—Experiments on the Localization of Developmental Factors in the Nemertine Egg. Jour. Exp. Zoöl., i.

The Basis for Taxis and Certain Other Terms in the Behavior of Infusoria.

By H. S. JENNINGS.

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THE BASIS FOR TAXIS AND CERTAIN OTHER TERMS IN THE BEHAVIOR OF INFUSORIA.

In the directed reactions of lower organisms we find repeated attempts by various authors to distinguish in one way or another two great classes of phenomena, and for these two classes different terms have been used. Thus ROTHERT[1] distinguishes two sorts of taxis, strophic and apobatic, depending on the manner in which they are brought about. Strophic taxis corresponds to the typical taxis or tropism of VERWORN or LOEB. It is conceived as brought about by a turning of the organism toward or from the source of stimulation, due to a difference in the intensity of action of the stimulus on the two sides or ends of the organism. Apobatic taxis is defined as produced by a backward movement at passing from one concentration to another.[2] The typical case for this is the reaction method of bacteria. ROTHERT includes under apobatic taxis the reaction of Paramecium and other infusoria to chemicals, etc., though for this his definition is inadequate, since the backward movement is only a comparatively unimportant detail of the behavior, and is often omitted.[3]

PFEFFER[4] rejects ROTHERT's terms, substituting for strophic taxis the word topotaxis, for apobatic taxis the word phobotaxis. MASSART[5] applies the term taxis only to the phenomenon called by ROTHERT strophic taxis, while the word phobism is proposed for the backward movement called by ROTHERT apobatic taxis.

DAVENPORT[6] makes in his discussion of the reactions to light a distinction corresponding to a certain extent with those already mentioned. He distinguishes reactions whose direction is determined by the direction of the rays of light, from reactions whose direction is determined by differences in intensity of illumination. The former he calls phototaxis, the latter photopathy. Photopathy is held to be due to the fact "that increased brightness causes a movement forwards, that a diminution of brightness causes a movement backwards, or *vice versa*, thus resulting in an accumulation of the organisms in the darker or lighter parts of the field." (l. c. p. 210.) Phototaxis on the other hand "includes that active migration of organisms whose direction is determined by that of the rays of light" (p. 180). In elongated organisms DAVENPORT thinks that phototaxis is due to the difference in intensity of illumination of the two sides of the organisms (p. 209), while in Amœba he thinks it possible that the direction taken by the ray in passing through the organisms is the determining factor (p. 210). YERKES[1] holds, with HOLT and LEE, that both phenomena are due in some way to the intensity of the light, and classes as taxis those reactions in which the direction of movement is determined by orientation, while -pathy includes reactions in which the movement "is not definitely directed through the orientation of the organism." This distinction doubtless indicates the real observational source of the attempt to separate two great classes of reactions, and does not attempt to make the definition outrun the known facts.

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[1] *Flora*, **88**, 1901, p. 393.

[2] *Rothert*, *l. c.* p. 393.

[3] See the account by the present author, this Journal, **14**, 1904, pp. 458-460.

[4] Pflanzenphysiologie, Bd. 2, 1904, p. 755.

[5] *Annales de l'Institute Pasteur*, 1901, p. 25.

[6] Experimental Morphology, vol. 1, 1897, p. 180.

Kinesis is a term which seems to have been first used by ENGELMANN[2] for the increase or decrease of activity produced by certain agencies. The fact that certain bacteria increase or decrease movement in the light he called photokinesis. ROTHERT (*l. c.*, p. 374) accepted the term kinesis for such changes in the amount of activity produced by chemicals, calling this chemokinesis. LOEB[3] had observed similar phenomena and applied to them the German word *Unterschiedsempfindlichkeit.* GARREY,[4] working with LOEB, later substituted the term kinesis for the German word. The term *Unterschiedsempfindlichkeit*, signifying literally sensitiveness to differences, might well include much more than a mere increase or decrease of activity; it could properly embrace all reactions due to a change in the intensity of action of a stimulating agent. GARREY's use of the term kinesis seems a little uncertain, since he seems in one place (p. 292) inclined to consider it equivalent to DAVENPORT's -pathy, thus including directed reactions to changes in intensity, while in general he seems to limit it to increase or decrease of activity, in the sense in which it was employed by ENGELMANN and ROTHERT. GARREY uses the term in contradistinction to tropism, which is defined in accordance with the well known views of LOEB.

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[1] Mark Anniversary Volume, 1903, p. 361.
[2] *Arch. f. d. ges. Physiol.*, **30**, 1882.
[3] *Arch. f. d. ges. Physiol.*, **54**, 1893, p. 81.
[4] *Am. Journ. Physiol.*, **3**, 1900, p. 29.

Terms ending in -metry have been contrasted with taxis, especially by STRASBURGER[1] and OLTMANNS,[2] to distinguish those features of the reactions that are determined by differences in the intensity of the stimulating agent. Photometry is employed by STRASBURGER to signify especially the phenomenon that some organisms move toward the source of light in a certain intensity of illumination, while they move away from it at a higher intensity. By OLTMANNS photometry is used of any reactions due to differences in the intensity of light; "movements produced by light of different intensity could fittingly receive the name photometric movement" (OLTMANNS, *l. c.*, p. 190).

Thus we find various attempts to distinguish by one criterion or another two great classes of reactions. On the one hand we have apobatic taxis (ROTHERT), phobotaxis (PFEFFER), phobism (MASSART), kinesis (ENGELMANN, GARRY and others), -pathy (DAVENPORT), -metry (STRASBURGER and OLTMANNS); in these orientation is not a marked feature. On the other hand we have tropism or taxis proper (LOEB, DAVENPORT, MASSART, VERWORN and others), strophic taxis (ROTHERT), topotaxis (PFEFFER); in these orientation is a marked feature. What is the precise basis for this distinction into two classes?

In the behavior of infusoria (ciliates and flagellates) it is possible to distinguish clearly the two classes of phenomena on which this distinction is based, and to determine the real nature of the difference.[3] In both classes the cause of reaction is, in the infusoria, some change in the relation of the organism to the environment. It is usually, or perhaps universally, a change in the intensity of action of a stimulating agent on the body as a whole or on its most sensitive portion. The organism, having been in one condition, passes to another, and it is the transition that acts as the effective stimulation. In one of the two classes of reactions to be distinguished, the effective stimulation is due to the fact that as the organism progresses from one region to another it meets different conditions, and to the changes thus caused it reacts. This is the case under natural conditions with the reactions to mechanical stimuli, to heat and cold, to chemicals, to osmotic pressure, and to light when passing to a region of greater or less intensity of illumination. The cause of reaction is analogous to that in our own case in passing from a region of moderate temperature to a hot or cold region. The differences determining reaction are here arranged along the axis of the course, so that it is the backward or forward movement that brings them into action. The reaction is a change of movement such as to carry the organism successively in many directions—a series of trial movements. As soon as one of these movements carries the organism away from the stimulating agent—that is, in such a direction that the changes to which it subjects the organism lead toward the optimum instead of away from it—the reaction ceases, since the cause for it has ceased. The organism therefore continues in that direction. The position of the body has little or no effect on the production of the change that causes stimulation, or on the release from stimulation. The organism might retreat from the stimulating agent backward or forward or sideways; in the one case as in the other it would be relieved of the stimulating changes. The different individuals may swim away directly or obliquely, their

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[1] *Jena. Zeitschr. f. Naturw.*, **12.** 1878. [2] *Flora*, **75**, 1892, p. 183.

[3] In the effects of the electric current the usual reaction method is in the Ciliata interfered with by the forced cathodic reversal of the cilia, —a factor not par-

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alleled under any other conditions. As a result of this interference the reaction of the ciliates to the electric current takes in many respects a different character from the rest of their behavior. I wish therefore to emphasize the fact that the general relations set forth in the text do not apply to the reactions of the ciliates to this agent. Save for the forced cathodic reversal of cilia the response to the electric current would fall in our second class of reactions, as it actually does in the flagellates and rotifers.

various paths crossing at all sorts of angles; the only requirement is that the path shall on the whole lead away from the greatest (or least) intensity of the stimulating agent. Hence when many specimens react in this way their paths need not be parallel, and as a rule no marked orientation results. When a common orientation of many individuals does occur, it is produced through "exclusion,"—through the fact that under the given conditions movement in any direction but one causes the changes which act as effective stimuli, so that all are forced to move in that direction. The behavior of Oxytricha in reacting to heat, as shown in Fig. 7 of the author's paper on Reaction to Heat and Cold[1] will serve as a type for this reaction method. It is, so far as the infusoria are concerned, the basis for apobatic taxis (ROTHERT), phobotaxis (PFEFFER), and -pathy (DAVENPORT), and might farther be classed, from certain points of view, as kinesis or -metry.

The second class of reactions includes those in which the changes that act as stimuli are brought about by a swerving toward one side or the other, while the movements in the axis of progression have no such effect. In the infusoria movements from side to side are of course a regular part of the locomotion. In the reactions to the effects of water currents, of centrifugal force, of gravity, and of light rays coming from one side, the lateral movements of unoriented animals induce marked changes. In a water current or under the action of a centrifugal force, or of gravity, lateral movements meet with less resistance in one direction, greater resistance in another direction, and these changes of resistance act as stimuli. In light coming from one side the sensitive anterior end is more illuminated as the organism swerves towards one side; less illuminated as it swerves toward the other, and these changes act as stimuli. The reaction is the same as in the first class; the organism changes its course by a series of trial movements. It continues these movements till it comes into a position in which it is no longer subjected to the changes that act as stimuli. Such a position is found only when the axis of the course coincides with the direction of action of the impinging force; in other word, when the organism is oriented. The anterior end will be directed toward the source of the stimulating agent or away from it, according as it is the decrease or increase in intensity that is the effective stimulus[1]. The transference of the organism along the axis of progress has in these cases no effect on the relation of the organism to the stimulating agent, so that so far as this reaction is concerned backward progression might take place as well as forward. Under these stimuli a certain orientation of the organism, not a certain direction of progress, is the essential result of the reaction. This class of reactions forms the basis, so far as the infusoria are concerned, for taxis proper (MASSART, DAVENPORT, etc.), strophic taxis (ROTHERT), and topotaxis (PFEFFER).

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[1] Carnegie Institution of Washington, Publication 16, 1904, p. 16.

Thus in the first class of reactions the essential result is a certain direction of progression—toward a region of greater or of less intensity—while in the second class the essential result is the orientation. But in both classes the nature of the stimulus is the same and the reaction is the same. The stimulus is some change in the relation of the organism to the surrounding conditions—a change in the intensity of action of some agent. The response is a motor reaction that consists of a series of trial movements. The response continues in each case until the effective stimulus comes to an end, then the usual motion is resumed. In the first class this does not produce orientation (save sometimes by "exclusion"), because stimulation comes to an end without it. In the second class it does produce orientation, because stimulation does not come to an end without it. The difference between the two classes depends on the peculiar difference in the distribution of the stimulating agent, not upon different ways of reacting on the part of the organism. The reaction is by "trial" movements continued till a cessation of the effective stimulation is brought about; this will lead to orientation or not, just as the conditions require.

H. S. JENNINGS.

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[1] For details as to this relation, see this *Journal*, **14**, 1904, pp. 470, 472, and 478; Carnegie Institution, Publication 16, 1904, p. 60.

MODIFIABILITY IN BEHAVIOR.

I. BEHAVIOR OF SEA ANEMONES.

BY

H. S. JENNINGS.

A thorough study of the modifiability of reactions to external stimuli in lower organisms seems at present one of the great desiderata in the study of animal behavior. Recent work has been devoted largely to the study of sharply defined forms of reaction and to the discovery of conditions under which these forms appear in the typical way. As a result there is a widespread impression that the behavior of lower organisms is composed of invariable reflexes, occurring always in the same way under the same external circumstances. This is far from the truth and leads, as it seems to the writer, to a fundamentally false conception of the nature of animal behavior. Inner states and changes are fully as important in determining behavior as are external stimuli, modifying fundamentally the reactions which the latter produce. The present studies are devoted to an analysis of some of these modifying factors; in other words to some of the inner factors in behavior.

The study of the behavior of sea anemones herewith presented was made possible by a stay at the Carnegie Research Laboratory at the Tortugas. I am under great obligations to the Carnegie Institution and to the director of the laboratory, Dr. A. G. Mayer, for opportunity to carry on the work, and for supplying every facility that could assist it. The Tortugas laboratory furnishes an ideal situation for carrying on such investigations. An indefinite number of species of sea anemones and corals can be procured at a few moments notice, and they live as well in the laboratory as in the sea, since the water becomes cooler instead of warmer when brought into the house.

I. CHANGES IN BEHAVIOR DUE TO VARYING STATES OF METABOLISM.

Nagel ('92) and Parker ('96) have shown that the food reaction of actinians toward weak stimuli becomes changed on repetition of the stimulation. A Metridium or an Adamsia at first readily takes filter paper soaked in dilute juice of crab meat. But after this has been fed several times in alternation with pieces of meat, the reaction to the filter paper becomes slower, and finally ceases, while the meat is taken as readily as before. Torrey ('04) shows that in Sagartia the state of hunger or satiety determines largely the reaction to small solid bodies. A very hungry Sagartia readily swallows inert bodies, such as filter paper and sand grains, while a fairly well fed one rejects these, though it takes meat. Has this effect of hunger and satiety any connection with the changes observed by Nagel and Parker, or are these of a different character? What relation have they to the changes due to experience in higher animals? The whole problem of the changes induced in behavior by changing metabolic states is one of the greatest importance for an understanding of the adjustment or regulation produced in behavior. I have attempted to study this matter carefully in a number of sea anemones, and to distinguish modifications due to this cause from those which result from other factors.

Stoichactis Helianthus.

This large sea anemone has often a disk 10 to 15 cm. in diameter. This is covered closely with short tentacles of uniform size, about 8 mm. in length.[1] Stoichactis is voracious; it is usually when captured ready to take large quantities of crushed crab appendages. To three specimens I fed piece by piece nearly all of three good sized ghost crabs (Ocypode). The food reaction depends on contact with the meat itself—that is, on chemical stimuli in combination with contact. Hard parts of the crab, or other indifferent objects, are usually not taken, though in rare cases even filter paper is swallowed.

Food is taken in the following way: If a piece of crab's leg, with some of the flesh exposed, is placed on the disk of a hungry specimen, the tentacles immediately surrounding it (including many not in contact with it) begin suddenly to wave back and forth. After an instant this usually ceases, and all is absolutely quiet for a few seconds. Then the movement begins again. All the tentacles that in their waving motion come in contact with the food, bend over against it and shrink, in such a way as to hold it down against the disk. Now that portion of the disk bearing the food begins to sink inward, by a folding of the surface. The mouth, which may be 4 or 5 cm. distant, begins to open, and the walls of the esophagus protrude from the mouth as large bladdery lobes. The region between the mouth and the food body begins to contract, the tentacles borne here collapsing and almost completely effacing themselves. By this contraction the mouth and food approach each other, the intervening region disappearing. Meanwhile other parts of the disk swell and their tentacles become plump and enlarged; this appears to be a secondary phenomenon due to the squeezing of the internal fluid from the contracted region to other parts. The esophageal lobes increase in size, becoming 2 to 4 cm. long, and half as thick; they extend toward the food, finally reaching it. By the contractions and expansions already mentioned the mouth may be moved from the center of a disk 10 cm. in diameter to within 1 cm. of the edge. By this time mouth and food may be hidden beneath the surface of the contracted disk, though in other cases they lie on the surface in plain view. Now the esophageal lobes extend over and around the food, while the tentacles progressively withdraw from it until the food body is lying on the contracted portion of the disk, completely covered by the esophageal lobes. Next that part of the disk beneath the food withdraws, involving an enlargement and further displacement of the mouth, till there is nothing beneath the food body, and it is pressed by the esophageal lobes into the internal cavity. The whole reaction is thus very complex.

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[1] For photographs of the disk of Stoichactis, see Duerden, 1902, Pl. 1.

Twenty or more pieces of crab, including entire large appendages, may thus be successively taken, till the body of the anemone has become a mere stretched sack full of crab appendages. But in the later reactions of a series the process of food-taking becomes much slower, the animal seeming to become gradually satiated. The food may be taken by the tentacles and held for a long time before it is finally moved to the mouth. In other cases the tentacles do not react for some minutes, the food lying on the disk undisturbed, until finally it is slowly taken. Sometimes there is an interesting combination of the positive food reaction and the negative reaction (to be described later). The food is taken by the tentacles and carried very slowly to the mouth, in the way above described, while the mouth opens and the esophageal lobes are protruded. But when the food body reaches the lobes, or sometimes before, the process stops. The food is released by the tentacles, and is finally carried away and rejected, in the way to be described. Finally, when the animal seems fully satiated, the piece of crab meat may be rejected as soon as it comes in contact with the disk. But after one or more pieces have been rejected one may sometimes see another piece accepted. The internal state is in a condition of most unstable equilibrium, and may easily incline toward the positive or the negative reaction.

Thus it is clear that in Stoichactis the reaction to a given stimulus is by no means a set, invariable property of the organism, but depends on the state of the internal processes. To the same stimulus we may get a quick positive reaction or a quick negative reaction; a slow and deferred positive reaction or a combination of the positive and negative reactions.

Peculiar effects are observed when several pieces of meat are placed at the same time on different parts of the disk. If the animal is hungry all are carried to the mouth; the entire disk folds inward and the pieces are swallowed simultaneously or successively. I have seen six pieces, placed as far apart on the large disk as possible, thus ingested. When the animal is less hungry the results are different. In some cases, when two pieces of meat are placed on the disk, one is swallowed while the other is rejected. If the rejected piece is again placed on the disk after the first piece has been disposed of, it will sometimes be swallowed.

Adding new pieces while swallowing is in progress often produces interference. Thus, in one case two pieces of meat, *a* and *b*, were placed near opposite edges of the disk. Both began to approach the mouth in the usual food reaction. Now two new pieces, *c* and *d*, were placed near the edge midway between *a* and *b*. Thereupon the reaction to *a* and *b* ceased, while *d* was transported to the edge of the disk (about 2 cm.) and dropped off. Now the food reaction was resumed, *a*, *b* and *c* traveling toward the mouth.

Piece *d* was now replaced on the disk. The reaction to the other pieces was suspended, and *d* was carried to the mouth. Here it came against the middle of the esophageal lobe that was extending toward *a*,—in such a way that *d* could not well be ingested without a rearrangement of the lobes. Thereupon *d* was again carried away from the mouth and once more dropped over the edge of the disk. The other pieces were now successively swallowed. Piece *d* was readily swallowed when given to another specimen.

The Rejecting Reaction.—After Stoichactis has become satiated, it rejects food, as we have seen. The rejecting reaction presents a number of points of much interest. By this same reaction the disk is kept clean when débris falls upon it. If a mass of waste matter of any sort (as a mass of dead plankton or a quantity of sand) is placed on the disk of Stoichactis, measures are set in operation which result, within ten or fifteen minutes, in removing this material and leaving the disk free. The behavior in bringing about this result is complex and the operation may be accomplished in more than one way.

The tentacles bearing the débris or the rejected food body collapse, becoming thin and slender, and lying flat against the disk. At the same time the disk surface in this region begins to stretch, separating the collapsed tentacles widely. As a result the waste mass is left on a smooth, exposed surface, the tentacles here having practically disappeared—though under usual conditions they form a close investment almost completely hiding the surface of the disk. Thus the waste mass is fully exposed to the action of waves or currents, and the slightest disturbance in the water washes it off. Under natural conditions this must usually result in an immediate removal of the débris. If this does not occur at once, often the region on which the débris is resting begins to swell, and becomes a strongly convex, smooth elevation, thus rendering the washing away of the mass still easier.

But the process may go much farther. If the débris is not removed in the way just described, new reactions set in. If the mass is nearer one edge of the disk this edge usually begins to sink, while at the same time the tentacles between the edge and the waste object collapse and practically efface themselves. Thus a smooth, sloping surface is produced and the waste mass slides off the disk. If this does not occur at once, after a little time the region lying behind the mass (between it and the center of the disk) begins to swell, producing a high, rounded elevation, with tentacles plump and swollen. The waste mass is now on a steep slope, and is bound soon to slide down and over the edge. Sometimes by a continuation of this process the entire disk comes to take a strongly inclined position, with the side bearing the débris below. Often one portion of the edge of the disk after another is lowered in this way, till all the waste matter has been removed. The disk then resumes its horizontal position, with nearly flat or slightly concave surface.

Sometimes the edge bearing the débris cannot be lowered, owing to the fact that it is almost against an elevation in the irregular rock to which the anemone is attached. In this case, after perhaps an attempt to bend the edge downward, the part between the edge and the waste body swells and rises, rolling the mass toward the center, while at the same time the region between it and the center sinks down. The sinking continues till it reaches the opposite edge, so that the mass is rolled across the disk to the opposite side and there dropped off the disk. The process is slow, often taking fifteen minutes to half an hour.

The rejecting reaction is characterized by great flexibility and variability. The débris or refused food sets in operation certain activities; if these do not remove the source of stimulation, other activities are induced until one is successful.

Thus in Stoichactis the same stimulus—crab's meat—may in the same individual produce sometimes the long train of activities resulting in the ingestion of food; in other cases the complicated and variable behavior resulting in rejection, in still others a combination of the two. The deciding factor is internal—the condition of the metabolic processes.

Aiptasia.

Two species of Aiptasia were studied. One was Aiptasia annulata Les.; the other a smaller and darker species, with shorter tentacles, which I have been unable to identify with certainty. I shall call it Aiptasia No. 2. Both came from the moat surrounding Fort Jefferson. Rather small specimens, with columns 4 to 10 cm. in length, were used in most of the work.

The species of Aiptasia are relatively active and quick-moving anemones. Especially is this true of Aiptasia annulata. If the tip of one of the long tentacles is touched, the whole disk and column shrinks with a sudden quick contraction, reminding one of the rapid contraction of a medusa. To the eye all parts of the body appear to contract at once. Often the disk and column have contracted strongly before the actual contraction wave has made any apparent progress from the tip of the long tentacle to the disk. Certainly in this animal the general contraction does not appear to be due to a spreading of an actual contraction wave from one part of the animal to another, through the actual pulling of one region upon the neighboring one, as it does in Hydra, and according to Torrey ('04), in Sagartia. On the contrary, there seems certainly to exist some rapid method of conduction, suggesting nervous action.

In Aiptasia annulata the use of India ink indicates the presence of cilia driving a current away from the mouth and toward the tip of the tentacles, as in Metridium.

Aiptasia annulata usually takes crab meat or filter paper soaked in the juices of such meat, but refuses neutral bodies, such as plain filter paper or sand. Aiptasia No. 2, on the other hand, is usually prepared to swallow readily balls of plain filter paper and other small neutral bodies, as well as crab meat. This furnishes opportunity for some interesting comparative experiments.

Food is taken in the following way: If a small object comes in contact with a tentacle it adheres to the surface, and the tentacle contracts strongly, the whole animal usually contracting at the same time. Then the tentacle bends over and places the food with considerable precision on the mouth. The tentacles near by likewise bend over and are applied to the food body, holding it down against the mouth. This happens even when the body is quite neutral, as plain filter paper, so that the bending of the neighboring tentacles is clearly due to some influence transmitted from the one tentacle in contact with the body. The mouth now opens, the lips protruding a little and seizing the food, while the tentacles may release it and bend away. But sometimes the tentacles follow the food into the mouth and their tips remain enclosed for some time. The actual swallowing of the food is mainly due to the activities of the lips and esophagus; it may occur without any intervention of the tentacles, when the food is placed directly on the mouth. A piece of meat or filter paper may be completely enclosed by either species within ten seconds of the time it comes in contact with a tentacle.

With these two species of Aiptasia the experiments of Nagel and Parker, mentioned on page 448, were repeated and varied, with somewhat peculiar results. Pieces of crab meat and of filter paper (plain or soaked in juice of crab meat) were given alternately to the individual under experimentation. In Metridium and Adamsia, as we have noted, the animal soon comes to reject the filter paper, while still accepting the meat.

In Aiptasia annulata a typical experiment is as follows: The animal is fed alternately filter paper soaked in crab juice and crab meat. Both are taken readily till four pieces of each have been ingested. At the fifth piece of paper—the ninth piece of the whole series—the animal balks and rejects it. But it likewise rejects the immediately following fifth piece of meat! It has evidently lost its hunger, and refuses to take anything. This is the usual result with Aiptasia annulata.

In Aiptasia No. 2 plain filter paper (not soaked in crab juice) was given alternately with pieces of crab meat. In a typical experiment six pieces of filter paper and six of meat were taken in regular alternation. But the seventh piece of paper and the immediately following seventh piece of meat were rejected.

The results above given are the usual ones. But sometimes, though rarely, results are reached which are analogous to those attained in Metridium by Parker. Thus, in one case a specimen of Aiptasia annulata accepted the first piece of plain paper, but thereafter refused paper consistently, while accepting meat offered in regular alternation with it.

For all these results the following explanation suggests itself: The animals when hungry take both meat and filter paper; when satiated they take neither. Usually the tendency to take both ceases at the same point, but sometimes the reaction to the weaker stimulus (filter paper) cease before that to the stronger stimulus—as a higher animal that is not hungry may refuse most things, while accepting peculiarly tempting morsels.

If the degree of hunger is thus the determining factor, then it should be possible to produce the rejection of the filter paper by feeding meat alone. This turns out to be the case. Indeed, usually the rejection of filter paper may be induced more readily by feeding meat alone than by feeding the two alternately, or than even by feeding filter paper alone. Thus, two specimens of Aiptasia No. 2, which we will call A and B, living side by side, were both found to take plain filter paper readily. Then A was fed alternately meat and filter paper, while B was fed successive pieces of meat. After eight pieces had thus been fed to each, A still took filter paper (though slowly), while B refused it absolutely—though B would still slowly take a piece of meat. Thus B, through satisfying its hunger with meat, had come to reject filter paper, while A still accepted it after devouring several pieces. Apparently meat is more satisfying to sea anemones than is filter paper!

In another case a specimen of the same species was fed filter paper alone. It swallowed ten pieces in succession, till the body was puffed out with them, meanwhile ejecting some of the pieces already swallowed, in the intervals between the taking of new ones.

In Aiptasia annulata similar relations were found. The animal could be caused to reject filter paper soaked in crab juice much more readily by feeding it meat alone than by feeding soaked paper alone, or by feeding the two in alternation. A large number of comparative experiments were tried, showing this result to be general. It is therefore clear that the state of hunger or satiety is the essential factor in this behavior, in Aiptasia.

The experiments showed further that it is not the mere mechanical fulness of the digestive cavity that determines acceptance or rejection, but some change in the metabolic processes themselves. Filling the digestive cavity with filter paper does not have the same effect in producing rejection as does filling it with meat. Even when the cavity is so filled that pieces of paper are repeatedly disgorged, new pieces are readily taken. In Aiptasia No. 2, a piece of paper that has been disgorged after remaining some time in the cavity, is usually swallowed again immediately, if it is returned to the disk.

As the animal becomes less hungry the details of the behavior toward food bodies change greatly. In a hungry specimen, as we have seen, the food reaction is rapid, often requiring but ten to fifteen seconds. After several pieces of meat have been ingested the reaction of all parts becomes much slower and less precise. The tentacles touched by the food may not react at all for several seconds; then they bend in a rather languid way toward the mouth, while the surrounding tentacles may quite omit their reaction. The food body is not placed so accurately upon the mouth as in the hungry individual. At a further stage toward satiation, a piece of crab meat applied to the tentacles induces either no reaction at all or a straight withdrawal—a negative reaction; they may then bend back from the disk along the column. If the meat is placed directly on the disk, in contact with the mouth, the latter may very slowly open and in a languid way partly or entirely enclose the food, even when there is no reaction of the tentacles. The mouth is thus usually readier to give the food reaction than are the tentacles.

In this condition of approaching satiation some peculiar combinations and alternations of positive and negative reactions may be observed. In a specimen of Aiptasia No. 2 after five pieces of alternate meat and paper had been taken, another piece of paper was swallowed, then after one and one-half minutes this was disgorged. The disgorged piece lay on the disk for a few seconds, then the mouth opened and began swallowing it again. But after it was about half enclosed, it was again rejected. Now it was grasped again and partly re-swallowed, then again rejected. This performance was repeated once more before this piece of paper was definitely rejected. A fresh piece of paper presented immediately after was slowly swallowed, then in two minutes disgorged. The anemone presented exactly the spectacle which we should interpret in a higher organism as a struggle between desire and repugnance for the available food.

In another case a piece of meat was presented after six pieces had been swallowed. The tentacles reacted only very slowly, but finally deposited the piece of meat on the disk, and withdrew. The mouth opened part way, then closed again without ingesting the food. Later it opened again a very little and enclosed a minute shred of the meat between its lips. The piece was thus quietly held for ten minutes, when it was seen to be sinking imperceptibly. Fifteen minutes after it was given it was completely enclosed. Many other cases were seen of partial rejection and acceptance of the same piece of meat. At times after one piece has been rejected, another is accepted.

In Adamsia and Metridium, according to Nagel ('92) and Parker ('96), after the tentacles of a certain region of the disk have through repeated trials come to reject soaked filter paper, those of another part of the same disk will still carry it to the mouth. This shows clearly that a general lack of hunger on the part of the organism as a whole cannot be the only factor involved. In Aiptasia No. 2 I tried experiments to determine whether there was the same independence in the tentacles of different regions. Crab meat was given to the tentacles of the left side; these carried it to the mouth, where it was swallowed, the tentacles of the right side playing no part in the reaction. After the tentacles of the left side had taken five pieces they reacted very slowly, a piece of meat resting against them for several seconds before it was seized. When it was finally carried to the mouth, however, it was swallowed readily. The next piece of meat, not being seized at once by the left tentacles, was transferred to those of the right side. They seized it instantly and quickly carried it to the mouth. Thus it is clear that the experience of the individual tentacles plays some part in the behavior; either from fatigue or some other cause, tentacles frequently stimulated gradually lose the tendency to respond. The fact that this result is produced by meat, the purest form of food, seems to indicate that fatigue may be the cause.

But the rest of the experiment indicates that this plays only a minor part in the change of behavior. After a short rest the giving of food to the tentacles of the left side was resumed. They continued to carry it slowly and with much delay to the mouth, where it was very slowly swallowed. After taking four more pieces, the tentacles of the left side absolutely refused to carry any more food to the mouth. The mouth had now almost ceased taking food when directly applied to it, though after some minutes the food was finally ingested. Now a piece of meat was given to the tentacles of the right side, which had only reacted once, and that more than fifteen minutes ago. Yet they behaved in exactly the same way as did the others, refusing to react at all, save by hanging back from the disk along the column.

Thus it is clear that the animal is a unit so far as hunger and satiety are concerned. If the satiety has arisen through the activity of the tentacles of one side, the tentacles of the other side are equally affected by it. It is the general progress of metabolism that is the chief factor in determining the reactions to food.

As Torrey ('04) has already noted for Sagartia, the reactions of satiated sea anemones differ in many other ways from those of hungry specimens. The well fed animal reacts much less readily and strongly to simple mechanical shock. If touched with a needle the well fed individual of Aiptasia either does not react at all, or contracts very slightly, while the hungry specimen reacts suddenly and powerfully. A slight disturbance in the water has no effect on the well fed individual, while the hungry one contracts strongly. To chemical stimuli the same relations apply. A much stronger solution of any given chemical is required in order to produce contraction in the well fed individual, as compared with the hungry one. The bearing of such facts on quantitative determinations in reaction work is evident. If we should attempt to determine the strength of a given chemical which causes contraction in Aiptasia, we should obtain totally different results, according as we used specimens that were very hungry, moderately hungry, or thoroughly satiated. No "normal" concentration for causing reaction could be determined for even a single given specimen, for the state of metabolism, and with it the tendency to react, is continually changing.

It is, of course, clear that the change due to varying metabolic states cannot be interpreted alone as a general increase or decrease of sensitiveness. Much more significant is the complete qualitative change in the nature of the reaction to a certain stimulus, due to this cause, which we have seen both in Stoichactis and in Aiptasia.

2. ACCLIMATIZATION TO STIMULI.

Sea anemones show acclimatization to stimuli in the same way as do the protozoan Stentor and many other low organisms. A light stimulus that is not injurious may cause at first a strong reaction, then on repetition produce no reaction at all, or a very slight one. This is easily shown with Aiptasia annulata in the following way: A specimen is selected with outspread disk close beneath the surface of the water. From a height of about 30 cm. a drop of water is allowed to fall on the water surface just above the disk. At once the animal contracts strongly. Waiting till it has expanded again, another drop is allowed to fall in the same way. As a rule there is no reaction to this or to succeeding drops. Sometimes there is a response to the first two or even three drops, but usually there is no reaction after the first one. A slight reaction of a different sort, that often comes on later, will be mentioned in the next section.

Experiment shows that the failure to respond is practically universal if the drops fall three minutes or less apart. With drops five minutes apart there is still marked evidence of acclimatization, though irregularities appear. With drops falling at intervals of more than five minutes I was unable to satisfy myself with certainty that acclimatization occurs.

Related to the present subject are changes in the reaction to light. Aiptasia annulata is very sensitive to light, expanding in darkness, but contracting after a few seconds when exposed to strong light. In ordinary daylight the animal remains contracted for some hours, but after such a period most specimens extend in spite of the light. In comparative darkness the animals direct the disk toward the source of light, through a contraction on the side of the column exposed to the light. After remaining undisturbed for a long time in an aquarium that is fairly well lighted, the animals give up their orientation with respect to the strongest source of light; with less light they retain it.

3. REACTIONS MODIFIED AS A RESULT OF THE PAST EXPERIENCES OF THE ORGANISM.

Under this head will be considered all positive changes in reaction, due to former stimuli or former reactions of the organism, aside from those due to changes in metabolism.

We have already described certain cases belonging here. In the reaction by which the disk is kept clean in Stoichactis we find that a mass of débris on the disk causes first one reaction, then another, till one of these or a combination of several rids the animal of the stimulating agent (see p. 451). In this case either the continuation of the same stimulus, or the fact that a certain reaction has been given, induces a new reaction, without change in the external conditions.

A similar phenomenon is often seen in the experiments with falling drops of water, described above. To the first drop the animal responds by a sudden sharp contraction, then to a considerable number of drops there is no response. Now if the drops continue, the animal usually begins to shrink slowly away from the region where the drops are falling, so that in the course of time the disk has been withdrawn some distance below the surface, though no decided reaction has occurred to any one stimulus. These facts are precisely parallel to those which I have described in a previous paper (1902, p. 50) for the infusorian Stentor.

More marked changes result when the animal is stimulated by light strokes of a rod. At the first stroke on the disk Aiptasia contracts strongly. It then extends in the same direction as before. When it is fully extended the stimulus is repeated. The animal responds in the same way as at first. This is usually continued for about ten or fifteen stimulations, the animal each time extending in the same direction as at first. But at length, when stimulated anew, the animal contracts, bends over to one side, and extends in a new direction. Under natural conditions, where stimulation at every extension would usually be due to some fixed object, this would of course put an end to the series of stimuli. If, however, the stimuli are still continued after each extension, the animal repeats for a number of times the extension in the new direction, then finally turns again and tries a new position.

This may be repeated many times. But in the course of time the reaction becomes changed in a still different manner. The anemone releases its foothold and moves to a new region. This result I have not succeeded in attaining by striking the animal with a rod each time it extends; the time required is evidently to be measured in hours. But obstructions may be so placed that every time the animal extends, the disk strikes against a solid body. In such a case it is usually found after a few hours that the animal has moved to a new region.

Thus to the same stimulus when repeated many times the anemone reacts first by contraction, then by turning repeatedly into new positions, then by moving away. The phenomena are parallel to those described by the present author ('02) for the infusorian Stentor, and by Wagner ('05) for Hydra. Beyond doubt other stimuli would here, as in Hydra and Stentor, produce the same series of reactions.

In the behavior just described there are at times certain phenomena which bear a striking resemblance to the formation of new habits. Aiptasia annulata frequently extends its body in most awkward turns, the column retaining an irregular and crooked form. This is evidently due to its method of life. The animal lives in irregular crevices and crannies beneath stones or in the hollows of the coral reefs. In order that its disk may protrude into the free water, it is often compelled to extend in the irregular way mentioned, and to retain the crooked forms thus reached. When removed from the natural habitat it still retains these irregularities of form and action. The lower part of the column may stand at right angles to the upper part, or there may be permanent S-shaped bends, or still more irregular forms. It would appear that these must have arisen as a result of the way in which it extends in its natural habitat. The peculiar methods of extension found in given individuals could then hardly be characterized otherwise than as habits, the peculiarities of form being the structural correlates of the habits.

In searching for experiments that would test the possibility of the formation of new habits in sea anemones, the following suggested itself. It should be possible to produce new habits in Aiptasia by so arranging the surroundings as to compel the animal to extend in a new way whenever it extends, and to retain the new form thus induced. If the animal when thus compelled by obstacles to extend in a new direction, still extends in the same direction after the obstacles are removed, one would be inclined to hold that a new habit had been formed.

I supposed that this result would require a long period of time. But some preliminary experiments showed it to be attained, in some cases, with such absolute ease as to raise the doubt whether we have here anything that can be called habit formation. Thus an individual attached to a plane horizontal glass surface was bent in extension far over to the left. Stimulating it repeatedly, it contracted at each stimulation, then bent, in extending, again to the left. This continued for fifteen stimulations, one succeeding another as soon as the animal had become fully extended. At the next contraction the animal turned and bent over to the right. Now when stimulated it contracted as before, then bent regularly, in extending, over to the right. It seemed to have acquired a new habit—bending to the right instead of to the left.

Attentive examination showed that when the animal contracted in response to stimulation, the concave side of the column contracted a little more than the rest, so that that side remained a little shorter. In other words, the animal did not take on an entirely symmetrical structure, but the region which was most contracted in extension remained most contracted also in the contracted animal. Now on expanding, all parts extended more or less proportionately to their extension in the contracted animal, so that the original curved form was regained. In other words, the structural conditions resulting in the curved form were not really given up even in contraction, and were only made evident when extension occurred.

If the animal was compelled by repeated strong stimulation to contract maximally in all parts, then in extension there was no greater tendency to bend in the direction previously occupied than in any other. And in about half the individuals this result followed (after once the first habitual position found in nature had been given up) even after a single stimulation, so that there was no indication of anything like the formation of a new habit.

What is the interpretation to be given to the numerous cases in which bending in a certain direction when extended *does* induce, in the way set forth above, bending in the same direction on a new extension? Is this the formation of a habit? It is certainly a condition of affairs that gives the same result as habit formation. The anemone might indeed be looked upon as a sort of structural model, illustrating the principles on which habit formation might occur. A certain action (extension in a certain direction) leaves structural peculiarities, persisting even in the intervals of action (in the contracted state), which result in a repetition of the same action. Is not this the picture that we commonly make for ourselves of the real nature of habit formation? In the sea anemone this seems to occur in a relatively gross way, but it appears difficult to point out any difference in principle between this and habit formation. If the persisting structural peculiarities were of such a nature as to be hidden from observation, there would be no ground for hesitation in calling these phenomena the formation of habits. There can hardly be doubt that the striking individual peculiarities of action and structure, described above, have arisen in precisely this way, so that it plays the part taken by habit formation in higher animals.

It would be well if the study of this matter could be extended to the same individual for a long time, beginning with a young, still regular, specimen, compelling it to live in a position where it would have to extend in a definite irregular way. In this way the development of the structural correlates of the habits (?) could doubtless be observed.

The facts may be summed up for the anemone as follows: Performance of a certain action involves the assumption of certain structural conditions. These conditions persist in a slight degree even in the intervals between the actions. At a new action they show their influence by causing it to take place in the same way as the former one. This gives the same results as what we are accustomed to call habit.

4. GENERAL AND COMPARATIVE.

The sea anemones are among the lowest of the Metazoa, and their behavior, when compared with that of most other animals, is of a very simple character. Yet it is evident that even in these low organisms the reaction to a given external stimulus depends upon many things beside the nature of the stimulus itself. Varying states of metabolism induce totally different reactions to the same stimulus, one state producing the long train of actions looking toward the ingestion of food, another inducing the equally long and variable chain of activities resulting in rejection. The same factors cause marked changes in reaction to other stimuli than possible food. Past stimuli received and past reactions performed likewise determine the reaction to a given external condition, resulting sometimes in a cessation of reaction, in other cases in a complete change in its character. Certain simple conditions produce a tendency in the organism to perform more readily an act previously performed (bending, on extension, in a certain direction).

Examination of the conditions under which the animals live shows clearly that all the usual reactions and modifications of the reactions are such as to assist in adapting the organism to its environment. In other words, they aid the physiological processes of which the organism is the seat. Aiptasia annulata, for example, lives in crevices beneath and among stones or coral rocks. It is, of course, evident that its food reactions maintain its metabolic processes, which would necessarily cease in their absence, that the rejecting reaction keeps the surface clean, so that respiration may take place uninterruptedly, and obstacles or injurious substances be avoided. The transformation of the food reaction into the rejecting reaction after the animal is satiated with food is of course as much to the interest of the sea anemone as it is to that of higher animals. If the food reaction were an invariable reflex, occurring whenever food is present, without regard to internal conditions, the results would be disastrous. The fact that the very hungry animal will take indifferent bodies that would otherwise be rejected is of course likewise adaptive; as Torrey ('04) remarks "substances with a very small food value must be of some importance to a starving polyp although they would not be desirable as food to a well nourished animal."

The tendency of Aiptasia to remain in the dark and to contract when strongly lighted keeps it in the crevices where it finds protection for its soft body. The fact that it faces and bends toward the lighted side keeps its tentacles and disk directed toward the entrance to the crevice, where food may be captured; if they were directed toward the darkest part of the crevice little or no food would be obtained. While the contraction under light is protective, it would result, if continued indefinitely by a lighted polyp, in starvation; we find that after a considerable period of light the animal extends. In correlation with its life in irregular crevices or under stones we find that Aiptasia does not take any definite position with reference to gravity, as some other anemones do. Such a reaction would render its usual habitat impossible. The tendency to react by a quick contraction when there is a slight disturbance in the water is undoubtedly protective. Yet such a disturbance when not followed by an attack from its author is not harmful and the animal under such circumstances quickly resumes its usual behavior, even though the disturbance continues. But such a disturbance maintained indefinitely would result in loss of opportunity for obtaining food, and the animal after a time shrinks gradually away from such a disturbed region. Injurious stimuli, interfering with the natural physiological processes of the polyp, cause contraction—the animal withdrawing from the field of action for a time. But this continued indefinitely would result in a loss of food and doubtless other injurious effects. We find that the animal has recourse then to extension in another direction, and finally to creeping away and establishing itself elsewhere. Located in an irregular crevice, we find that the polyp extends in various directions, until it finds a direction in which its disk and tentacles are unimpeded in their spreading to form a trap for prey. It then continues to extend in this manner, even though this may require the body to bend at right angles or to take other irregular forms. It continues to extend in this manner even when removed from its irregular crevice, and the body is found to have become structurally modified, so that a collection of Aiptasias shows many crooked and zigzag shapes, each being an adaptation to the crevice in which the animal lived. The formation of such habitual methods of extension can be imitated and modified in the laboratory.

All together, the activities and their modifications are clearly such as to directly adjust the organism to its environment, enabling the physiological processes to continue under all sorts of conditions. It has become the fashion to neglect such facts, but they fairly force themselves on the attention of the careful student of the behavior, and their existence can hardly be held to be accidental. To remove such an organism to the artificial conditions of the laboratory and then endeavor to understand its behavior is like dissecting an internal organ out of the body and trying to understand its functions when thus separated from the other structures with which it interacts. Almost everything the animal does has a direct relation to something in its usual environment, and when cut off from this environment, its activities are likely to become unintelligible. One can hardly resist the belief that the fact that these activities do assist the physiological processes of the organisms has determined their selection and retention from among other possible activities.

This adaptation and adaptive modifiability of behavior in sea anemones and their relatives has not been explicitly set forth in most works dealing with their reactions. Yet when other careful accounts of behavior in such organisms are analyzed we can discover such relations as clearly as in Aiptasia. Let us look for example at the cases of Hydra, studied by Wagner ('05), and of Cerianthus, as described in the classical papers of Loeb ('91). It will be found instructive to consider the conditions on which the retention of a certain position depends. Hydra and the sea anemones tend as a rule to retain a position at rest, with the foot attached and head free. This usual position is often said to be due to a reaction to gravity, or to contact, or to some other simple stimulus. But when we examine into the matter closely, we find that it is not an entirely simple one. Let us take first the case of Hydra. Suppose the animal is placed on a horizontal surface with head downward and foot upward. It does not retain this position, but bends the body, placing the foot against the bottom, releases its head, and straightens upward. Aiptasia shows the same reaction. In neither of these animals is the reaction due to a tendency to keep the body in a certain position with reference to gravity, for both keep the body indifferently in any position with reference to the pull of gravity, provided that the foot is attached and the disk and tentacles can be spread freely. To what then is the reaction due? Evidently there is a tendency to keep the foot in contact with a surface, for the body of the inverted Hydra is bent till the foot comes in contact. There is likewise a tendency to keep the head free, for it is released. But this is not all, for now the body is straightened, then the tentacles are spread out symmetrically in all directions. It is clear that the reaction is directed toward getting the organism into a position that may be called "normal," and this normal position has various factors—attachment of foot, freedom of head, comparative straightness of body, and tentacles outspread.

Suppose now that our Hydra has reached this position, and all the conditions remain constant; is this sufficient? We find that it is not. If the conditions remain so constant that no food is obtained, the Hydra becomes restless and changes the position of its body repeatedly, though still retaining its attachment by the foot. Later even this is given up, and the animal, of its own internal impulse, quite reverses the position attained through the "righting reaction." It now bends the body, attaches the head, and releases its foot, thus bringing it back into the inverted position.

Is this because the irritability of head and foot have become reversed, so that the head now tends to remain attached, the foot free? Apparently not, for no sooner has the animal taken the inverted position than it draws its foot forward and now performs the "righting reaction" again, so that it stands once more on its foot. These alternations of behavior are repeated, and we find that by this means the animal is moving from place to place (see Wagner, 1905, Fig. 3).

It seems clearly impossible to refer each of these acts or the whole behavior to any particular present external stimulus. An internal state—hunger—drives the Hydra to move to another region, and these different opposite acts are the means by which another region is reached. Each phase of the locomotion is evidently partly determined by the fact that a certain other phase has just been performed, partly by the general state of hunger. The same behavior is shown by Hydra under continued injurious stimuli of different sorts.

In speaking of righting reactions, it is often said that the organism is *forced* by the different irritabilities of diverse parts of the body to take a certain orientation with reference to gravity or to the surface of contact (see for example Loeb, 1900, p. 184). The facts just brought out (taken from Wagner) show that we cannot in Hydra consider this orientation forced, save in the general sense that all things which occur may be considered forced—including of course the behavior of man. Man takes sometimes a sitting position, sometimes a standing one, sometimes a reclining one, depending upon his "physiological state" and past history, and the facts are quite parallel for Hydra. So far as objective evidence shows, the behavior is not forced in Hydra in any other sense than it is in man. Both organisms take that position which seems best adapted to the requirements of their physiological processes; these requirements vary from time to time.

In the sea anemone Cerianthus the conditions for staying in a certain position are somewhat more complex than in Hydra, according to the account given by Loeb (1891). Cerianthus is usually found in an upright position, inhabiting a tube made of mucus and imbedded in the sand. If placed head downward in a test tube, it rights itself in the same way as Hydra and Aiptasia, freeing the head, bringing the foot into contact, and straightening the body. But in Cerianthus Loeb showed clearly that gravity plays a part in the behavior. If the animal is placed on its side on a wire screen of large mesh, it bends its foot down through the meshes, lifts up its head, and takes its usual position with reference to gravity. If now the screen is turned over, the animal again directs its head upward, its foot downward—as a human being under similar circumstances would do if possible. It may thus weave itself in and out through the meshes.

But to be in line with gravity, with head above and free, is not the only requirement for Cerianthus. Loeb found that it would not remain indefinitely in this position on the wire screen, as it does in the sand. After a day or so it pulls its foot out of the wire and seeks a new abode. Only when it can get the surface of the body in contact with something, as is the case when it is imbedded in the sand—in its natural habitat—is it at rest. If this condition is fulfilled, the requirement of the usual position in line with gravity may be neglected. Loeb found that when the animal is placed in a test tube, so that its body is in contact with the sides, it remains here indefinitely, *even though the tube is placed in a horizontal position* (Loeb, 1891, p. 54). The head is bent upward, but the body remains transverse to the direction of gravity.

Examples of the fact that a certain orientation with reference to gravity is not a rigid requirement even in animals that usually or at times react to this agent, are common among sea anemones and other lower organisms. Thus, Torrey ('04) shows that Sagartia, though it usually maintains an upright position, may ofttimes take a position on the surface film, with head downward. In the rejecting reaction of Stoichactis, described on p. 451, we have clearly a reaction with reference to gravity, though one which even the most sanguine could hardly denominate a fixed tropism. The situation "waste-matter-on-the-disk-not-removed-by-the-first-(usual)-reaction" is responded to by taking such a position with reference to gravity as results in removing the waste; then the reaction to gravity ceases. This is somewhat analogous to the reaction to gravity described by Bohn (1903) in the hermit crab. While investigating a shell which it may adopt as a home if fitting, this animal takes up a certain position with reference to gravity—namely, with the body on the steepest slope of the shell, and head downward; it then turns the shell over and ceases to react with reference to gravity. Of a different but equally significant character are the variations shown in the reactions to gravity by the low acelous flatworm Convoluta, as described by Bohn ('03b) and Gamble and Keeble ('03). Under conditions that are favorable Convoluta remains on the surface of the sand. But when the sun becomes hot, or when the tide rises, so that the animal is likely to be washed away, it becomes "positively geotropic," going downward in the sand, where it is protected. When the tide falls again Convoluta becomes "negatively geotropic," thus reaching the surface of the sand, where it obtains food and carries on its usual activities. These alternations of reaction become a fixed habit with Convoluta, so that when removed to an aquarium it still goes downward at high tide, upward at low tide, though the conditions surrounding it remain constant; it may thus be used for a time as an in-door tide indicator. Gradually, however, when removed for a long time from the influence of the tides, this alternation of reactions to gravity ceases, showing it to be a true habit, resulting from individual experience. Many other instances of reactions to gravity, of the most diverse sorts and variable character, could be given. Gravity affects organisms in many diverse ways—determining the distribution of internal substances of differing specific gravity, causing differences in the ease of movements in diverse directions, inducing strains or pressure in unaccustomed parts of the body when an unusual position is taken—indeed, influencing the life processes in almost every detail. Any of the points at which it comes in contact with the life processes may serve as the basis for a reaction, so that we find behavior induced by relations to gravity in different organisms to be of the most diverse character. We have been assured by various writers that the reaction to gravity must be explained in the same way in all cases, but this is evidently said rather in the capacity of a seer or prophet, than in the capacity of a man of science whose conclusions are inductions from observation and experiment.

Returning to Cerianthus, we find, according to Loeb, that even the usual position in line with gravity and with sides in contact, does not satisfy the animal indefinitely, if left quite undisturbed. If it secures no food it again leaves its place and seeks another region.

Thus in order that Cerianthus may remain quiet in a given position, a considerable number of conditions should be fulfilled, constituting the usual, and perhaps what we may call the "normal" state of affairs for this animal. These conditions are the following: (1) The foot should be in contact; (2) the head should be free; (3) the body should be straight; (4) the axis of the body should be in line with gravity, with the head above; (5) the general body surface should be in contact; (6) food should be received at intervals. If these conditions are largely unfulfilled, the animal becomes restless, moves about, and finds a new position. But no one of these conditions is an absolute requirement at all times, unless it be that of having the head free. In the wire screen the animal remains for a day or two in the required position with reference to gravity, even though foot and body surface are not in contact. In the horizontal tube it remains with foot and surface in contact, though the body is not straight nor in line with gravity. If all conditions are fulfilled save that of food, the animal remains for a time, then moves away.

Clearly, the holding of any given position depends, not on the relation of the body to any one or two sources of stimulation, but on the proper maintenance of the natural physiological processes of the organism. The actinian does not always maintain a certain position with relation to gravity, nor does it always keep its body straight, nor its foot in contact, nor its body surface in contact. It does not at all times receive food. It may remain quiet for considerable periods with one or more conditions lacking. The organism tends on the whole to take such a position as is most favorable to the unimpeded course of its natural physiological processes. Certain usually required conditions may be dispensed with provided other favorable ones are present. The behavior, like that of higher animals, represents a compromise of the various needs imposed upon the animal by its physiological processes.

Examination of the literature shows that throughout the Cœlenterates there is a similar dependence of behavior on the progress of the internal physiological processes, particularly those of metabolism. The state of metabolism decides whether Hydra shall creep upward to the surface or shall sink to the bottom (Wilson '91), how it shall react to chemical and to solid objects (Wagner '05), whether it shall remain quiet in a certain position, or shall reverse this position and undertake a laborious tour of exploration. In the sea anemones it determines, as we have seen, even the details of long trains of reaction. The state of the metabolic processes appears to be the most important determining factor in the behavior of Cœlenterates.

The same dependence of behavior on the internal physiological processes is found in other groups, even in those much lower than the Cœlenterates—the Protozoa, and particularly the Bacteria. This is brought out especially in some of the work of Engelmann. A number of examples of this relation will be given in the paper which follows the present one, so that they may be omitted here. The fact that in higher animals behavior depends largely on hunger and satiety is, of course, so well known that it need not detain us.

The relation of behavior to the internal physiological processes, of which we have given some examples in the foregoing pages, is manifestly of the greatest significance for the understanding of behavior. The facts adduced show directly that in many cases the determining factor in reactions to stimuli is not the anatomical configuration of the body, taken in connection with simple laws of conduction, but is the relation of the action of the external agent to the internal processes. The problem presented by the fact that the same stimulus, in the same intensity, applied to the same part of the body, produces qualitatively different and even opposite results, depending on the inner metabolic states, seems not to have received the attention it deserves. It evidently places marked difficulties in the way of a simple mechanical conception of the reflex process, based merely on the anatomical structure of the organism. The internal physiological state determines in some way which of various courses within the body the transmitted stimulus shall follow and what organs it shall arouse to activity. The organism cannot be looked upon as a static structure, on which external agents must act in a simple invariable way. *The organism is a process*, and some of the chief determining factors in behavior are given by the relation of the internal to the external processes. As the internal processes change, the reaction to external agents changes correspondingly. We find that reactions which assist the existing internal processes are continued or repeated, while those which oppose them are changed. This gives one of the chief bases for the regulatory character of behavior, as I shall attempt to set forth in farther detail in the paper which follows the present one. The metabolic processes, while the most striking of those taking place in the lower organisms, are of course not the only ones occurring in animals. An immense number of other processes are in progress, and the relation of external agents to these processes may and does equally determine behavior. This gives the phenomena of behavior their complexity, preventing them from being in relations of *simple* dependence on external agents, as they are often represented of late. Such a view quite underestimates the difficulty of the problem of behavior. The dependence on external agents exists, but is complex, and can usually not be predicted without a knowledge of the present internal state of the organism—this depending on its past history and the course of its various internal processes.

It would of course be more convenient if the problems of behavior were as simple as they are often proclaimed to be. Work revealing their complexity is naturally not received with the acclaim that greets the announcement that all these things are simple and easy. But if our object is really to obtain control of the vital processes, then we must face them in all their complexity. To control animal behavior it is necessary to study animal nature, in much the same way that it is necessary to study human nature in order to control human behavior. It is necessary to know the past history of the organisms, and what is going on within them, in order to predict what they will do. He who expects even the lower animals to behave always in certain simple invariable ways when acted upon by the various forces of nature has many disappointments in store, when he comes to make a thorough study of the matter. The internal modifying conditions must be made the object of deliberate and extended investigation in lower animals as well as in higher ones, before the study of behavior can be placed on a really scientific basis.

LITERATURE CITED.

Bohn, G., '03.—De l'évolution des connaissances chez les animaux marins littoraux. Bull. Institut Général Psychologique, No. 6; 67 pages.

'03.—b. Les Convoluta roscoffensis et la théorie des causes actuelles. Bull. Mus. d'Histoire Naturelle, pp. 352–364.

Duerden, J. E., '02.—Report on the Actinians of Porto Rico. Bull. U. S. Fish Com., vol. xx, second part, pp. 323–374, 12 pl.

Gamble, F. W., and Keeble, F., '03.—The Bionomics of Convoluta roscoffensis, with Special Reference to Its Green Cells. Quart. Journ. Micr. Sci., vol. xlii, pp. 363–431.

Jennings, H. S., '02.—Studies on Reactions to Stimuli in Unicellular Organisms. IX. On the Behavior of Fixed Infusoria (Stentor and Vorticella), with Special Reference to the Modifiability of Protozoan Reactions. Amer. Journ. Physiol., vol. viii, pp. 23–60.

Loeb, J., '91.—Untersuchungen zur physiologischen Morphologie der Thiere. I. Ueber Heteromorphose, pp. 48–59.

'00.—Comparative Physiology of the Brain and Comparative Psychology. 309 pages. New York.

Nagel, W., '92.—Das Geschmacksinn der Actinien. Zoöl. Anz., Bd. xv, S. 334–338.

Parker, G. H., '96.—The Reactions of Metridium to Food and Other Substances. Bull. Mus. Comp. Zoöl. at Harvard Col., vol. xxix, pp. 107–119.

Torrey, H. B., '04.—On the Habits and Reactions of Sagartia davisi. Biol. Bull., vol. vi, pp. 203–216.

Wagner, G., '05.—On Some Movements and Reactions of Hydra. Quart. Journ. Micr. Sci., vol. xlviii, pp. 585–622.

Wilson, E. B., '91.—The Heliotropism of Hydra. Amer. Natural., vol. xxv, pp. 413–433.

THE METHOD OF REGULATION IN BEHAVIOR AND IN OTHER FIELDS.

BY

H. S. JENNINGS.

The results set forth in the preceding paper, together with certain other relations found in the behavior of lower organisms, that have been detailed in previous papers by the present writer, suggest a certain point of view in regard to the general method of regulation or adjustment in organisms. Everywhere in the study of life processes we meet the puzzle of regulation. Organisms do those things which advance their welfare. There are some exceptions, but this is certainly true in a general survey. If the environment changes, the organism changes to meet the new conditions. If the mammal is heated from without, it cools from within; if it is cooled from without it heats from within, maintaining the temperature that is to its advantage. The dog which is fed a starchy diet produces digestive juices that are rich in the enzyms which digest starch, while under a diet of meat it produces juices rich in proteid digesting substances. When a poison is injected into a mouse, the mouse produces substances which neutralize this poison. If a part of the organism is injured, a rearrangement of material follows till the injury is repaired. If a part is removed, it is restored, or the wound is at least closed up and healed, so that the life processes may continue without disturbance. Regulation constitutes perhaps the greatest problem of biology. How can the organism thus provide for its own needs? To put the question crudely, how does it know what to do when a difficulty arises, so as to overcome this difficulty? It seems to work toward a definite purpose. In other words, the final result of its action seems to be present in some way at the beginning, determining what the action shall be. In this the action of living things appears to contrast with that of things inorganic. It is regulation of this character that has given us the theories of vitalism. By these theories the principles controlling the life processes are held to be of a character essentially different from anything found in the inorganic world.

Nowhere is regulation more striking than in the behavior of organisms. Indeed, the processes in this field have long served as the prototype for regulatory action. The organism moves and reacts, on the whole, in ways that are advantageous to it. If it gets into hot water, it takes measures to get out again, and the same is true if it gets into excessively cold water. If it enters an injurious chemical substance, it at once changes its behavior and escapes. If it lacks material for its metabolic processes, it sets in operation movements which secure such material, suspending these movements when the lack is fully supplied. If it lacks oxygen for respiration, it moves to a region where oxygen is found. If injured it flees to safer regions. In innumerable details it does those things which are good for it, and this is as true of the Protozoan as of the Metazoan. It is plain that behavior depends largely on the needs of the organism, and is of such a character as to satisfy these needs. In other words, behavior is adjustment or regulation.

There seems no reason to think that regulation in behavior is of a different character from that found elsewhere. But nowhere else is it possible to perceive so clearly how regulation occurs. In the behavior of the lowest organisms we can see not only what the animal does, but precisely how this happens to be regulatory. The method of regulation lies open before us. This method is of such a character as to suggest the possibility of its application to other fields; in other words, it suggests a possible general explanation of the method of regulation. This suggestion the present paper attempts to develop.

In the lower, unicellular, organisms where we can see just how regulation occurs, the process is as follows. Anything injurious to the organism causes changes in behavior. These changes subject the organism to new conditions. As long as the injurious condition continues, the changes in behavior continue. The first change in behavior may not be regulatory, nor the second, nor the third, nor the tenth. But if the changes continue, subjecting the organism successively to all possible different conditions, a condition will finally be reached that relieves the organism of the injurious action, provided such a condition exists. Thereupon the changes in behavior cease, and the organism remains in the favorable condition. The movements of the organism when stimulated are such as to subject it to various conditions, one of which is selected.

This method of regulation is found in its purest form in unicellular organisms, such as Paramœcium and Stentor. Yet it occurs also in higher organisms, and indeed is found in a less primitive form throughout the animal series, up to and including man. When we ourselves, or other animals, are confronted with a difficulty for which neither experience nor inherited tendency has furnished us with a direct method of relief, the only recourse is to this same method of regulation. We perform movements which subject us to various conditions, till one is found that relieves the difficulty. We call the process searching, testing, trial, and the like. In the lowest and highest organisms the injurious condition acts as a stimulus to produce many movements, subjecting the organism to various conditions, one of which is selected.

In connection with this method of behavior three questions arise, which are fundamental for the theory of regulation. First, How is it determined what shall cause the changes in behavior that result in new conditions? Or why does the organism change its behavior under certain conditions, not under others? Second, How does it happen that such movements are produced as result in more favorable conditions? Third, How is the more favorable condition selected; what is this selection and what does it imply? Our first and third questions may indeed be condensed into one, which involves the essence of the regulatory process: Why does the organism choose certain conditions and reject others? This selection of the favorable conditions and rejection of the unfavorable ones presented by the movements is perhaps the fundamental point in regulation.

It is often maintained that this selection is precisely personal or conscious choice, and that behavior cannot be explained without this factor. Personal choice it evidently is, and in man it is often conscious choice; whether it is conscious in other animals we do not know. But in any case this does not remove it from the necessity for analysis. Whether conscious or unconscious, choice must be determined in some way, and it is the province of science to inquire as to how this determination occurs. To say that rejection is due to pain, acceptance to pleasure, or to other conscious states, does not help us, for we are then forced to inquire why pain occurs under certain circumstances, pleasure under others. Surely this is not a haphazard matter! There must be some difference in the conditions to induce these differences in conscious states (if they exist) and at the same time to determine the differences in behavior. We are therefore thrown back upon the objective processes occurring. Why are certain conditions accepted, others rejected? This is essentially what has often been called the pleasure-pain problem.

Such facts as are set forth in the preceding paper give us a basis for an objective answer to this question. Organisms are not static structures; processes of complicated character are in continual progress within them. Among these the processes of metabolism are most prominent. Ostwald ('02) has emphasized the point that one of the chief characteristics of living matter is the fact that processes are occurring with much energy within it. The organism is a complex of processes. In the preceding paper we have seen that the reactions of organisms to external agents depend largely on the relation of the action of these agents to the internal processes.

Let us examine certain cases of this dependence in the simplest organisms—bacteria and protozoa. The green Paramœcium bursaria requires oxygen in its metabolic processes. While swimming about it comes to a region where oxygen is lacking. It reacts by turning away and going in some other direction. The white Paramœcium caudatum does the same, and so also do many bacteria. All require oxygen in their metabolic processes; lack of oxygen interferes with these processes, and they react to such a lack by changing their movement and going elsewhere. But there are some bacteria that do not require oxygen in their metabolic processes. When these come to a region lacking oxygen they do not react, but keep on and enter this region. In many of these anaërobic bacteria oxygen is known actually to interfere with the physiological processes. When these bacteria come to a region containing oxygen, they change their movement and go elsewhere. In Paramœcia and the bacteria that require oxygen, this does not occur (unless the amount of oxygen rises above the optimum). In all these cases, whenever there is interference with the metabolic processes, the organism reacts by turning away, otherwise it does not. In the reactions of these creatures with reference to light and darkness we see the same thing. In the green Paramœcium bursaria, in Euglena, and many other green infusoria, light assists the metabolic processes, while lack of light interferes with them, and the same is true of the so-called purple bacteria. All of these organisms react on coming to a region of darkness by turning away and going elsewhere. In the colorless Paramœcium caudatum and in the colorless bacteria ordinary light does not affect the metabolic processes, so that there is no interference with these processes in darkness, and we find that they do not react on reaching a dark region, but enter it readily. For all these organisms, colored and uncolored, light may be made so intense that it does interfere with the physiological processes, as is shown by the fact that the processes stop, the organisms dying. To such light all react by turning away—including even the colorless Paramœcium caudatum. We find in all of these cases that the animal reacts by turning away when there is interference with the physiological processes, and does not so react unless there is such interference.

In some cases the relation between behavior and the effect of an agent on the physiological processes is marvelously precise. Thus, Engelmann ('82) proved that in Bacterium (Chromatium) photometricum the ultra red and the yellow-orange rays are those which most favor metabolism (assimilation of carbon dioxid, etc.). When a microspectrum is thrown on a preparation of these bacteria, they are found to react in such a way as to collect in precisely the ultra red and the yellow-orange. The reaction consists in a change of behavior—a reversal of movement—at the moment of passing from the ultra red or the yellow-orange to other parts of the spectrum, while passing in the opposite direction produces no such effect. Bacteria are not in nature subjected to spectral colors in bands, so that there has been no opportunity for the production of this correspondence between behavior and favoring conditions through the selection of varying individuals.

What is the explanation of these facts? Why does the infusorian or the bacterium shrink back from darkness or the region containing no oxygen? As a matter of fact, it requires the light or the oxygen for the continuance of its metabolic processes, and it does not shrink back from a region lacking them unless it does need them. But we have no reason to attribute to the bacterium any knowledge or idea of that relation. We do not need any purpose or idea in the mind of the organism, or any "psychoid" or entelechy to account for the change of behavior, for an adequate objective cause exists. We know experimentally that the darkness or the lack of oxygen interferes with the metabolic processes. This very interference is then evidently the cause of the change of behavior. When anything interferes with the internal processes, running with much energy, the energy overflows in other directions, resulting in changes in behavior. This statement is a mere generalized formulation of the facts determined by observation and experiment in the most diverse organisms.

Internal as well as external interference may cause the changes of behavior. If oxygen or other material for metabolism is lacking to such an extent as to interfere with the metabolic processes, the organism changes its behavior. In the sea anemones, as we have seen in the preceding paper, this condition induces the animal to change its position and start off on a laborious tour of exploration. The initiation of changes in movement through internal conditions gives the basis for the reactions which we call positive, as we shall see.

The answer to our first question is then as follows: The organism changes its behavior as a result of interference with its physiological processes.

Our second question was: How does it happen that such movements are produced as bring about more favorable conditions? This question we have already answered, so far as many lower organisms are concerned, in our general statement on page 474. The organism does not go straight for a final end. It merely acts—in all sorts of ways possible to it—resulting in repeated changes in the conditions. In this way a condition is after a time reached that relieves the interference with the internal processes.

The nature of the changes in behavior produced—the movements that occur in any given organism—depend on what may be called the "action system" of the organism. The animal, in other words, performs the movements that it is accustomed to perform, as determined by its structure and its past history. The essential fact is that interference with the internal processes causes a *change* in behavior. The mere fact of a change under these conditions tends in itself to be regulatory. The original behavior has brought on the interfering conditions, hence the best thing to do is to change this behavior. If the unfavorable condition still continues the behavior is changed again; this being continued, the organism is bound to escape from the interfering condition if it is possible to do so. In some cases the movements produced are, when considered by themselves, of a rather uniform character, yet are of such a nature as to subject the animal to many changes of the environmental conditions. This is the case for example in the reactions of such infusoria as Paramœcium, where the character of the movement is determined partly by structure, yet involves a continued change of relation to the outer conditions. In other cases the movements themselves are varied in character, the organism first reacts in one way, then in another, running through a whole series of activities, till one results in ridding the organism of the stimulating condition. This is the method of behavior seen in Stentor and in most higher organisms.

Our third question was: How does the organism select the more favorable condition thus reached? This question now answers itself. It was interference with the physiological processes that caused the changes in behavior. As soon, therefore, as this interference ceases, there is no further cause for change. The organism selects and retains the favorable condition reached merely by ceasing to change its behavior when interference ceases. This process is seen clearly in the behavior of such infusoria as Paramœcium.

It is perhaps fairly evident how reaction on the plan just described may result in the avoidance or rejection of sources of interfering stimuli; in other words, in the production of negative reactions. The matter of positive reactions should perhaps receive further elucidation.

In conditions that are completely favorable—so that all the life processes are taking place without lack or hindrance—there is no need, from the standpoint of regulation, for a change in behavior—for definite reactions of any sort. The most natural behavior on reaching such conditions, and that which is actually found as a rule among lower organisms, is a continuation of the activities already in progress. These activities have resulted in the favorable conditions, and there is no cause for a change. This we find exemplified in infusoria, bacteria, rotifers, and many other organisms, under most classes of conditions. A change in behavior takes place only when the activities tend to remove the organism from the favorable conditions; in other words, to produce interference with the life processes. Unfavorable stimuli, in these organisms, cause a change in behavior; favorable stimuli cause none. It is perhaps a general rule in organisms, high or low, that continued completely favorable conditions do not lead to definite reactions. Of course while the external conditions remain the same, the internal processes may change in such a way that these conditions are no longer favorable, and now the behavior may change. This frequently happens.

When the organism is not completely enveloped by favorable conditions, but is on the boundary, if we may so express it, between favorable and unfavorable ones, there is often a definite change in the behavior leading toward the favorable conditions—a positive reaction. To understand such reactions, we may start from the fact, already mentioned, that unfavorable internal conditions (as well as external ones) cause a change of behavior. It is a general fact, for example, that the animal whose metabolic processes suffer interference from lack of material—the hungry animal—sets in operation trains of activity differing from the usual ones. Interference with respiration, or an increase in temperature above that favorable for the physiological processes, has similar effects. This is indeed a general rule for all internal changes interfering with the usual physiological processes.

But the activities thus induced are in themselves undirected, save by structural conditions. There is nothing in the cause producing them to direct them with reference to external things. Let us suppose, however, that certain of these movements lead to a condition which relieves the interference with the internal processes. The cause for a change of behavior is now removed, hence the organism continues its present movement. But perhaps later—sometimes at the very next instant—this same movement may tend to remove the organism from the favorable condition—as when a Paramœcium in a heated preparation passes across a small area of water cooled to the optimum, and reaches the opposite side, or when a hungry organism comes in contact with food, which will be lost if there is further movement. Thereupon the cause for a change—interference with the life processes—is again set in operation, and the present movement is changed. Thus the animal changes all behavior that leads away from the favorable condition, and continues that which tends to retain it, so that we get what we call a positive reaction. The change of behavior is due in each case primarily to the unfavorable stimulation, internal or external. This style of behavior is seen with diagrammatic clearness in the free swimming infusoria. These animals continue their movements as long as they lead to favorable conditions, changing at once such movements as lead away. They thus retain favorable conditions by avoiding unfavorable ones; the positive reaction is seen to be, in a sense, a secondary result of negative ones.

We have a similar condition of affairs in the taking of food by Amœba. The animal moves forward with broad front, and comes in contact at a certain point on this front with a food body. Part of its movement is taking it away from the food, part toward the food. On coming in contact, all movement which takes it away is changed, only that being continued which keeps the animal in contact with the food. We have here then the same condition of affairs as in the infusoria—the selection of certain conditions through the rejection of all others.

This is perhaps the fundamental condition of affairs for organisms in general. In higher animals the positive, as well as the negative, reactions, have become complicated through the influences to be brought out later, so that this primitive condition is not evident. But the essential point is that unfavorable conditions are rejected as a result of the fact that they produce changes in behavior, and this results in the attainment and retention of favorable conditions. In negative reactions it is the new unfavorable external condition that is rejected, retaining the old favorable internal condition. In positive reactions it is the old unfavorable internal conditions that are rejected, retaining the new favorable external conditions. In both cases the impulse to change of movement comes from interference with the physiological processes—external interference in negative reactions, internal interference in positive reactions.

To sum up, in the lowest organisms we find individual adjustment or regulation on the basis of the three following facts:

1. Definite internal processes are occurring in organisms.
2. Interference with these processes causes a change of behavior and varied movements, subjecting the organism to many different conditions.
3. One of these conditions relieves the interference with the

internal processes, so that the changes in behavior cease, and the relieving condition is thus retained.

It is clear that regulation taking place in this way does not require that the end or purpose of the action shall function in any way as part of its cause, as some vitalistic theories hold. There is no objective evidence that a final aim is guiding the organism. None of the factors above mentioned appear to include anything differing in essential principle from such laws of causality as we find in the inorganic world.

Now an additional factor enters the problem. By the process which we have just considered, the organism reaches in time a movement that brings relief from the interfering conditions. This relieving response becomes fixed through the operation of a certain law which appears to hold throughout organic activities. This law may be stated as follows: An action performed or a physiological state reached, is performed or reached more readily after one or more repetitions, so that in time it becomes "habitual." The statement of this law just given is in reality not adequate, and it may be well to dwell upon it a moment, developing it farther, and pointing out some of the phenomena in which it is expressed. In previous papers, including the one immediately preceding the present, I have pointed out the fact that the behavior and reactions of an organism depend largely on "physiological states;" the same point has recently been emphasized by Bohn ('05). We may distinguish at least two great classes of physiological states—those depending on the metabolic processes of the organism, treated in detail in the preceding paper, and those otherwise determined. The physiological states of organisms change in accordance with certain laws. The changes in the metabolic states of course depend on the laws of metabolism. In the physiological states not directly dependent upon metabolism, but rather upon stimulation and upon the activity of the organism, such as are found in Stentor and Planaria (see Jennings, '04), we find certain fairly well defined laws of change that are of a peculiar character.

In the organisms just mentioned, and in many others, the following phenomena have been found. Under certain external conditions the organism reacts in a certain way. These conditions continuing, the organism changes its first reaction for a second, and then perhaps for a third and fourth. Later the same external conditions recur, and now the organism at once responds, not by its first reaction, but by the final one. This is illustrated for unicellular organisms by the case of Stentor, for higher metazoa by the behavior of certain crustacea, as described by Yerkes ('02) and Spaulding ('04). There are certain differences between the two cases, but they are not essential for our present purpose.

How does this condition of affairs come about? As we have set forth in previous papers ('04), the different methods of reaction under the same external conditions must be due to different physiological states of the organism. The "physiological state" is evidently to be looked upon as a dynamic condition, not as a static one; it is a certain way in which the bodily processes are occurring; it tends directly to the production of some change. In this respect the "law of dynamogenesis," propounded for ideas of movement in man, applies to it (see Baldwin, '97, p. 167); ideas must indeed be considered, so far as their objective accompaniments are concerned, as certain physiological states in higher organisms. The changes toward which physiological states tend are of two kinds. First, the physiological state, like the idea, tends to produce movement. This movement often results in such a change of condition as destroys the physiological state producing it. But in case it does not, then the *second* tendency of the physiological state shows itself. It tends to resolve itself into another and different state. State 1 passes to state 2, and this again to state 3. This tendency shows itself even when the external conditions remain uniform.

In this second tendency there manifests itself the important law of which we have spoken above. When a certain physiological state has been resolved, through the action of an external agent, or otherwise, into a second physiological state, this resolution becomes easier, so that in the course of time it takes place more quickly, and even spontaneously.

This may be illustrated from the behavior of the unicellular organism Stentor, as described in previous papers by the present writer ('02 and '04), as follows: When the animal is stimulated by the flood of carmin grains (or in any other way), this produces immediately a certain physiological state corresponding to that accompanying a sensation in ourselves. This state we may designate A. It at first produces no reaction. As the carmin continues or is repeated, this state A passes to a second state

B, producing a bending to one side. After several repetitions of the stimulus, the state B passes to the state C, producing a reversal of the cilia, and this finally passes to D, resulting in a contraction of the body. Each state must of course be different from the preceding one, because it produces a different result. The course of the changes in physiological states may then be represented as follows:

$$A \longrightarrow B \longrightarrow C \longrightarrow D$$

Now we find that after many repetitions of the stimulation the animal contracts at once as soon as the carmine comes in contact with it. In other words, the first condition A (direct result of contact) passes at once to the state D, and this results in immediate contraction:

$$A \longrightarrow D$$

It seems probable that the same series occurs as before, save that B and C are now passed rapidly and in a modified way, so that they do not result in a reaction, but are resolved directly into D. The process would then be represented as follows:

$$A \longrightarrow B' \longrightarrow C' \longrightarrow D$$

But whatever the intermediate conditions, it is clear that after the state A has become resolved, through pressure of external conditions, into the state D, this resolution takes place more readily, occurring at once after state A is reached.

The same law is illustrated in the experiments of Yerkes and Spaulding on association in crabs. In the experiments of Spaulding ('04) with hermit crabs, the introduction of the dark screen into the aquarium, and the diffusion of the juices of the fish, cause the animals to move about. In so doing they reach the dark screen, which induces, let us say, the physiological condition A. This leads to no special reaction. But this is followed regularly by contact with food, inducing the physiological state B, which is concomitant with a positive reaction. The physiological state A is thus regularly resolved into the state B. In the course of time this resolution becomes automatic, so that as soon as the state A is reached, it passes to B. The positive reaction concomitant with B is therefore given even though the original cause of B is absent. The actual number of physiological states which could be distinguished is of course greater than what we have set forth, but this does not alter the principle involved.

The law which we have just brought out may then be summed up as follows:

The resolution of one physiological state into another becomes easier and more rapid after it has taken place one or more times. Hence the behavior primarily characteristic for the second state comes to follow immediately upon the first.

The operations of this law are seen on a vast scale in higher organisms, where they constitute what we commonly call memory, association, habit, and the basis of intelligence. It has been shown to hold in a number of lower organisms, though in these the manifestations of this law are comparatively little known. Yerkes and Spaulding have demonstrated its applicability to Crustacea. The low acelous flatworm Convoluta evidently shows it clearly, since as we have seen in the preceding paper, it forms definite habits. It has even been demonstrated, as we have seen, in the protozoa, particularly Stentor and Vorticella. According to Hodge and Aikins ('95) a method of reacting thus developed lasted in Vorticella as long as five hours. In view of these facts, it is probable that the law is a general one and that it will be demonstrated in some form for other lower organisms. There seems to be no theoretical reason for supposing it to be limited to higher animals. The paucity of experiments fitted to test it is amply sufficient to account for the very slight knowledge we have of it in lower organisms.

To return then to the thread of our discussion: In virtue of this law of the readier resolution of physiological states after repetition, the final reaction of a trial series, relieving the organism of the interference with its physiological processes, is later reached more readily than at first, and in time becomes the immediate reaction to the interfering condition. Thus the change of behavior induced by interference of a certain sort has come to be of a perfectly definite character, and all trial movements are omitted.

It is in this second stage of the process, when the relieving response has become set through the law above discussed, that an end or purpose seems to dominate the behavior. This end or purpose of course actually exists, as a subjective state called an idea, in man. Whether any such subjective state exists in the lower organism that has gone through the process just sketched we of course do not know. But some objective phenomenon, as a transient physiological state, corresponding to the objective physiological accompaniment of the idea in man, would seem to be required in the lower organism. The behavior in this stage is that which, in its higher reaches at least, has been called intelligent.

But so far as the objective occurrences are concerned there would seem to be nothing in even this later stage of behavior involving anything different in principle from what we find in the inorganic world. The only additional feature is this law of the readier attainment of a certain state or action after repetition. We have not attempted to state this law in an entirely adequate manner, but there would seem to be nothing implied by it that is specifically vital, in the sense that it differs in essential principle from what we find in the laws of causality as applied to the inorganic world. It certainly by no means requires in itself the action of any "final cause"—that is, of an entity that is at the same time purpose and cause. On the other hand, it undoubtedly does produce that type of behavior which has given rise to the conception of the purpose acting as cause. This conception is in itself of course a correct one, so far as we mean by a purpose an actual physiological state of the organism, determining behavior in the same manner as other factors determine it.

That regulation takes place in the behavior of many animals in the manner above sketched seems to the writer an established fact, and it appears to be perhaps the only clearly intelligible way in which regulatory behavior could be developed in a given individual.

But we are of course confronted with the fact that many individuals are provided at birth with definitely regulatory methods of reaction to certain stimuli. In these cases the animal is not compelled to go through the process of performing trial movements, with subsequent fixation of the successful movement. How are such cases to be accounted for?

If the regulatory methods of reaction acquired through the process sketched in the preceding paragraphs could be inherited, there would of course be no difficulty in accounting for such congenital regulatory reactions, or habits. It is perhaps not going too far to say that this possibility is not yet out of court, though opinion at present seems to be generally against it. Yet Semon ('04) in his recent valuable monograph on the phenomena allied to memory and habit, maintains the affirmative view, and presents evidence in favor of it. We are in the beginning of the study of such problems, and it can hardly be said that experiments of sufficient duration and precision have yet been tried to really test the matter. If the inheritance of regulatory reactions acquired after trial should be demonstrated, the process sketched above would give us a satisfactory general method for the development of regulatory behavior, in the race as well as in the individual. In the protozoa this difficulty of course does not exist; the acquirements of individuals may remain as acquirements of the race.

If such inheritance does not occur, then the existence of congenital definite regulatory reactions would seem to be explicable only on the basis of the selection of individuals having varying methods of reaction, unless we are to adopt the theories of vitalism. In the method we have sketched above, a certain reaction that is regulatory is selected, through the operation of physiological laws, from among many performed by the same individual. In what is called natural selection, the same reaction is selected from among many performed by *different* individuals—in both cases because it is regulatory—because it assists the physiological processes of the organism. The two factors must work in the same direction. "Intelligence" and natural selection are two analogous methods of selecting the adaptive reactions from among the possible ones; they must then work together, as Baldwin ('02) has so well pointed out. Which of the two factors is the essential one in producing congenital adaptive reactions we shall of course not attempt to decide, since no one knows.

We often find in organisms behavior that is not regulatory, or that is regulatory only in a very imperfect way. How are we to account for this? Without going into details, it is evident that there exist at least three general conditions that may result in non-regulatory behavior. First, the organism is formed of substance that is subject to the ordinary laws of physics and chemistry. Various physical and chemical agents may act directly upon this substance, producing results that are not regulatory. The fact that the relation of external processes to internal ones is one of the chief determining factors in producing reactions, of course does not exclude the possibility of the direct action of agents on the body substance. The operation of intense physical and chemical agents may injure or destroy the substance of which the organism is composed, and with it the organism, in spite of any reaction the organism can give. Second, the organism can perform only those movements which its structure permits. Often none of these movements can relieve the existing interference with the physiological processes. Then the organism can only try them, without regulatory results, and die. Examples of this are seen in the behavior of Paramœcium, or of Planaria when placed in heated water. Both animals perform practically all the reactions of which they are capable, before they succumb. Third, certain responses may become fixed, in the way sketched above, because under usual conditions they relieve the organism. Now if the conditions change, the organism can respond at first only by this fixed reaction, and if this does not relieve, the animal may be destroyed before a new regulatory reaction can be developed. This condition of affairs is widespread among animals.

All together, the regulatory character of behavior as found in many animals seems perhaps intelligible in a perfectly natural, directly causal way, on the basis of the principles brought out above. We may summarize these principles as (1) the selection by varied movements of conditions not interfering with the physiological processes of the organism; (2) the fixation of the movements by which the selected conditions were reached, by the law of the readier resolution of physiological states after repetition. Neither of these principles seem to contain anything specifically vitalistic, or opposed in principle to what we find in the inorganic world.

Is it possible that regulation is based on similar principles in other fields than behavior? Bodily movement is only one of the many kinds of activity that may vary, and variations of any of the organic activities may impede or assist the physiological processes of the organism. Is it possible that interference with the physiological processes may induce changes in other activities—in chemical processes, in growth, and the like—and that one of these activities is selected, as in behavior, through the fact that it relieves the interference that caused the changes?

There is some evidence for this possibility. Let us look for example at regulative changes in the chemical activity of the organism, such as we see in acclimatization to poisons, in responses to changes in temperature, or in the adaptation of the digestive juices to the food. What is the material from which the regulative changes may be selected? One of the general results of modern physical chemistry is expressed by Ostwald ('02, p. 366) as follows: "In a given chemical structure all processes that are possible, are really taking place, and they lead to the formation of all substances that can occur at all." Some of these processes are taking place so slowly that they escape usual observation; we notice only those that are conspicuous. But in its enzyms the body possesses the means of hastening any of these processes and delaying others, so that the general character of the action shall be determined by the more rapid process. Such enzyms are usually present in the body in inactive forms (zymogens), which may be transformed into active enzyms by slight chemical changes, thus altering fundamentally the course of the chemical processes in the organism.

It is evident that the organism has presented to it, by the condition just sketched, unlimited possibilities for the selection of different chemical processes. The body is a great mass of the most varied chemicals, and in this mass thousands of chemical processes in every direction—all those indeed that are possible—are occurring at all times. There is then no difficulty as to the sufficiency of the material presented for selection, if some means may be found for selecting it. The process which will relieve any unfavorable condition, if any such process is possible, is actually occurring in an infinitesimal way, and needs only to be hastened.

Further, it is known that interference with the physiological processes does result in many changes in the internal activities of the organism, as well as in its external movements. Intense injurious stimulation causes not only "excess" movements of the body as a whole, but induces marked changes in circulation, in respiration, in temperature, in digestive processes, in excretion, and in other ways. Such marked internal changes involve, and indeed are constituted by, alterations of profound character in the chemical processes of the organism. These chemical changes are sometimes demonstrated by the production of new chemical substances under such circumstances. Furthermore, it is clear that the internal changes due to interference with the physiological processes are not stereotyped in character, but varied. Under violent injurious stimulation respiration may become for a time rapid, then is almost suspended. The heart for a time beats furiously, then feebly, and there is similar variation in other internal symptoms.

Thus it seems clear that interference with the life processes does induce varied activities in other ways than in bodily movements, and that among these are varied chemical processes. There is then presented opportunity for regulation to occur in the same way as in behavior. Certain of the processes occurring relieve the disturbance of the physiological functions. There results then a cessation of the changes. In other words, a certain process or condition is selected through the fact that it does relieve.

There is much evidence that the law of the readier resolution of physiological states after repetition applies to other bodily processes as well as to behavior. The much readier induction of digestive trouble by a small quantity of a certain food, after a large quantity has once induced it is perhaps an example; many better ones are given by Semon ('05). If the analogy with behavior holds in this respect, there will be present at a later period certain fixed methods of chemical response, by which the organism reacts to certain sorts of stimulation—as by the production of a definite antitoxin when a certain poison is introduced. Definite organs or organisms will have left open to them only certain limited possibilities of variation—due to the development of something corresponding to the "action system" in behavior. Thus, in the pancreas there will not exist unlimited possibilities as to the chemical changes that may easily occur. Its "action system" will be limited perhaps to the production of varied quantities of certain enzyms—amylopsin, trypsin, etc. The proper selection of these few possibilities will then occur by the general method sketched. When digestion is disturbed by food that is not well digested, variations in the production of the different enzyms will be set in train, and one of these will in time relieve the difficulty, through the more complete digestion of the food. Thereupon the variations will cease, since their cause has disappeared. By still more complete fixation of the chemical response, through the law of the readier resolution of physiological states after repetition, an organ or organism may largely lose its power of varying its chemical behavior, and thus be unable to meet new conditions in a regulative way. A condition comparable to the establishment of a fixed reflex in behavior will result.

It is perhaps more difficult to apply the method of regulation above set forth to processes of growth and regeneration. Yet there is no logical difficulty in the way. The only question would be that of fact—whether the varied growth processes necessary do primitively occur, under conditions that interfere with the physiological processes. When a wound is made or an organ removed, is the growth process which follows always of a certain stereotyped character, or are there variations? It is, of course, well known that the latter is often the case. In the regeneration of the earthworm, Morgan ('97) finds great variation; he says that in trying many experiments, one finds that what ninety-nine worms cannot do in the way of regeneration, the one hundredth can. The very great variations in the results of operations on eggs and young stages of animals is well known. Removal of an organ is known to produce great disturbance of most of the processes in the organism, and among others, in the process of growth.

It appears then not impossible that in growth processes regulation may be brought about in accordance with the same principles as in behavior. A disturbance of the physiological processes results in varied growth activities. Some of these relieve the disturbance; the variations then cease, and these processes are continued. In any given highly organized animal or plant the different possibilities of growth will have become practically much limited, and it is only from this limited number of possibilities that selections can be made. In some cases, by the fixation of certain processes through the analogue of the law of the readier resolution of physiological states, the organism or a certain part thereof will have lost the power of responding to injury save in one definite way. Under new conditions this one way may not be regulatory, yet it may be the only response possible. This may result in the formation under certain conditions of such things as heteromorphic structures—a tail in place of a head, or the like, from a part of the body that is accustomed (in normal development) to produce such an organ. This would again correspond to the production of a fixed reflex action in behavior, even under circumstances where this action is not regulatory.

It appears to the writer that the method of form regulation recently developed in a most suggestive paper by Holmes ('04) is in essential agreement with the general method of regulation here set forth, and may be considered a working out of the details of the way in which growth regulation would probably take place along such lines.

It may be noted that regulation in the manner we have set forth is what, in the behavior of higher organisms, at least, is called intelligence. If the same method of regulation is found in other fields, there is no reason for refusing to compare the action to intelligence. Comparison of the regulatory processes that are shown in internal physiological changes and in regeneration to intelligence seems to be looked upon sometimes as heretical and unscientific. Yet intelligence is a name applied to processes that actually exist in the regulation of movements, and there is, *a priori*, no reason why similar processes should not occur in regulation in other fields. When we analyze regulation objectively, there seems indeed reason to think that the processes are of the same character in behavior as elsewhere. If the term intelligence be reserved for the subjective accompaniments of such regulation, then of course we have no direct knowledge of its existence in any of the fields of regulation outside of the self, and in the self perhaps only in behavior. But in a purely objective consideration there seems no reason to suppose that regulation in behavior (intelligence) is of a fundamentally different character from regulation elsewhere.

It is perhaps hardly necessary to point out the relation of the method of regulation in behavior here discussed to the process of "selection of overproduced movements," so ably set forth in Baldwin's well-known works ('97, '02). The account here given is based on this same process, but differs in a number of points which seem to the writer of fundamental significance for a proper understanding of the method of regulation. Baldwin has likewise made some suggestion as to the possibility of extending this point of view to other fields (Baldwin '02).

We may make a general statement of the features in the method of regulation set forth in this paper, as follows: The organism is primarily activity. It is the seat of many processes, of chemical change, movement, and growth; these are proceeding with a certain amount of energy. These processes depend for their unimpeded course on one another and on the relations to the environment which the processes themselves largely bring about.

When any of the processes are blocked the energy overflows in other directions, producing varied changes in chemical processes, movement and growth. Some of the conditions reached through these changes relieve the interference that was the cause of the changes. Thereupon the changes cease, since their cause has disappeared; the relieving condition is therefore maintained. After repetition of this course of events, the change which leads to relief is reached more directly, as a result of the law of the readier resolution of physiological states after repetition. Thus are produced finally the stereotyped and under certain conditions non-regulatory changes sometimes resulting from stimulation.

This method of regulation is clearly seen in behavior, where its operation is, in the later stages, what is called intelligence. Its application to chemical and form regulation is at present hypothetical, but appears probable.

LITERATURE CITED.

BALDWIN, J. MARK, '97.—Mental Development in the Child and in the Race. Methods and Processes. Second ed., 496 pages. New York.

'02.—Development and Evolution. 395 pages. New York.

BOHN, G., '05.—Attractions et Oscillations des animaux marins sous l'influence de la lumière. Institut Général Psychologique, Mémoires, i, 110 pages.

ENGELMANN, TH. W., '82.—Bacterium photometricum. Ein Beitrag zur vergleichenden Physiologie des Licht- und Farbensinnes. Arch. f. d. ges. Physiol., Bd. xxx, S. 95–124.

HODGE, C. F., AND AIKINS, H. A., '95.—The Daily Life of a Protozoan: a Study in Comparative Psycho-physiology. Amer. Journ. Psychol., vol. vi, pp. 524–533.

HOLMES, S. J., '04.—The Problem of Form Regulation. Arch. f. Entw.-mech., Bd. xviii, S. 265–305.

JENNINGS, H. S., '02.—Studies on Reactions to Stimuli in Unicellular Organisms. IX. On the Behavior of Fixed Infusoria (Stentor and Vorticella), with Special Reference to the Modifiability of Protozoan Reactions. Amer. Journ. Physiol., vol. viii, pp. 23–60.

'04.—Physiological States as Determining Factors in the Behavior of Lower Organisms. Contributions to the Study of the Behavior of the Lower Organisms, fifth paper, pp. 109–127. Carnegie Institution of Washington, Publ. 16.

MORGAN, T. H., '97.—Regeneration in Allolobophora fœtida. Arch. f. Entw.-mech., Bd. v, S. 570–586.

OSTWALD, W., '02.—Vorlesungen über Naturphilosophie. 457 pages. Leipzig.

SEMON, R., '04.—Die Mneme als erhaltendes Prinzip im Wechsel des organischen Geschehens. 353 pages. Leipzig.

SPAULDING, E. G., '04.—An Establishment of Association in Hermit Crabs, Eupagurus longicarpus. Journ. Comp. Neurol. and Psychol., vol. xiv, pp. 49–61.

YERKES, R. M., '02.—Habit Formation in the Green Crab, Carcinus granulatus. Biol. Bull., vol. iii, pp. 241–244.

DESCRIPTION OF A NEW SPECIES OF EARTHWORM (DIPLOCARDIA LONGA) FROM GEORGIA.

BY J. PERCY MOORE.

Forming part of a small collection of earthworms gathered by Mrs. T. W. Walker in Pulaski county, Georgia, and secured for the Academy through the interest of Mr. Joseph Willcox, are seven specimens of a hitherto unnoticed species of *Diplocardia*. Its most distinctive feature is found in the coexistence of the second gizzard and the first pair of spermathecæ in the same somite, in which respect it is unique among known species of the genus. In the presence of the gizzards in somites VI and VII it resembles *D. michaelseni*, but differs from that species in the possession of three pairs of spermathecæ. The latter character and numerous others which appear in the following description evidently ally it to the *communis* group, but the great number of segments, high level of the nephridial openings, form of the spermathecæ, etc., are diagnostic.

Diplocardia longa n. sp.

Size of *D. communis;* length in moderate extension up to 275 mm.; diameter at VII 5 mm., behind the clitellum 4 mm. Number of segments 270 to 330. Form slender, terete throughout; diameter increasing to VII, then diminishing to the clitellum which is slightly enlarged and prominent, then narrowing a little and remaining nearly uniform to near the end, where a slight club-shaped enlargement precedes a final shrinkage to the anal ring.

Prostomium very short, broad and nearly truncate, in most specimens scarcely projecting beyond the peristomium into which it is tenoned for about one-half the length of the latter. The exact form, however, differs in the several specimens; in some the two sides nearly meet within the peristomium; in others they are more nearly parallel and may be continued by grooves nearly to the posterior border of the latter, or they may merge into transverse grooves. Both prostomium and peristomium are much marked above with longitudinal wrinkles and the former has a deep ventral furrow.

The somites increase in length to VII, and then diminish to about one-half in the postclitellial region, after which they change but little till the posterior end is approached. Somites II and III are simple; IV is biannulate, with the furrow just behind the setæ and consequently nearer to the posterior margin; V has a median setigerous annulus partially cut off from the larger annulus. The remaining preclitellial segments are conspicuously triannulate, the middle or setigerous annulus being the narrowest but most prominent. Occasionally the first annulus is again subdivided into two. When the clitellum is present, as in most of the specimens, the segments of this region are smooth and undivided, dorsally at least, but in immature worms the annulation appears here also. In all but the most posterior of the postclitellial somites also the triannular structure is apparent, but is generally inconspicuous except in the more anterior ones or those strongly contracted.

The usual four pairs of setæ are present on all somites except the peristomium, a few preanal, and XIX, the latter of which lacks the ventral pairs only. All are strictly paired and strongly ventral in position, but the intervening spaces vary somewhat. On XVI the setal formula is $a\text{-}b < \frac{1}{4}\, a\text{-}a$, $a\text{-}a = 2\frac{1}{2}\, c\text{-}d$, $a\text{-}b = \frac{3}{5}\, c\text{-}d$, $b\text{-}c < a\text{-}a$, $d\text{-}d =$ about $\frac{2}{3}$ semi-circumference. Ordinary setæ stout, sigmoid, blunt-pointed, thickened distad of middle, the outer end slightly sculptured with a reticulum of fine lines giving to the setæ an appearance of being covered with delicate scales. Spermathecal copulatory setæ (the ventral ones on VII, VIII and IX) similar but with much bolder sculpturing and apparently unaccompanied by special glands. Penial setæ (the ventral ones on XVIII and XX) capillary, tapering, somewhat thickened within the body-walls, with slight sinuous curvatures, especially near the end, which may be slightly hooked. They are about twice as long, or somewhat more, than the ordinary setæ, and only $\frac{1}{3}$ to $\frac{1}{4}$ as thick. Near the end is a sculptured region marked with close transverse lines appearing as fine notches on the profiles, but the end itself for a considerable distance is smooth or longitudinally striated.

Dorsal pores begin at $\frac{\text{IX}}{\text{X}}$ or usually $\frac{\text{X}}{\text{XI}}$ and are conspicuous. The position of the nephridiopores is remarkable in being widely removed from the dorsalmost seta; while the former are situated at approximately the two ends of the transverse diameter of the body the latter are fully 15° to 30° below these points. Successive nephridiopores are usually alternately at a little higher and lower level and are on the extreme anterior borders of the somites—almost in the furrows—the first on III. Spermathecal pores are less easily found. They occur in line with seta *a* on the anterior part of somites VII, VIII and IX, and are consequently presetal and postseptal.

Though present in all but one, the clitellum is perfectly developed only in the largest specimen. It covers seven segments, XIII to XIX inclusive, and is completely annular and of uniform thickness on the first five, but interrupted or much thinner on the middle ventral region of the last two. On the posterior clitellial and several of the immediately succeeding segments occur certain papillæ and grooves utilized during copulation. Two pairs of papillæ, situated on the posterior part of XVII or on $\frac{\text{XVII}}{\text{XVIII}}$ and on the posterior part of XX or $\frac{\text{XX}}{\text{XXI}}$, are constant on all of the specimens; a third pair situated on XXI or $\frac{\text{XXI}}{\text{XXII}}$ is complete in one specimen, represented by a median papilla in one, by the left only in three specimens, by the right only in one, and is totally absent in the seventh, in which, however, indistinct thickened areas appear in the median field of $\frac{\text{XXI}}{\text{XXII}}$ and $\frac{\text{XXII}}{\text{XXIII}}$. In one specimen a small median papilla is present on XVII between those of the pair. When the papillæ overlap the contiguous borders of two segments, which is the normal condition, the affected furrows are obliterated ventrally. In most cases the first pair of papillæ are much the largest and the third the smallest, but in this respect also they are variable. All of the papillæ are low, broad disks of an irregular elliptical or sometimes circular form. The central portion is more translucent and either elevated or depressed above the more opaque, firmer rim which contains a circle of glands. The entire structure of these papillæ indicates that in addition to a secretory adhesive function of the rim the center acts as a true vacuum sucker. Just anterior and posterior to each pair of suckers transverse grooves usually extend across the venter and other shorter and less constant ones may occur. Very constant and conspicuous are a pair of longitudinal grooves reaching in the line of the ventral setæ from the middle of XVIII to the middle of XX. Each groove consists of three parts: a short anterior section reaching from the setæ of XVIII to the furrow $\frac{\text{XVIII}}{\text{XIX}}$, a longer middle section extending for the entire length of XIX, and a short posterior section completing the groove to the setæ of XX. All three sections are strongly curved, the anterior and posterior with the convexity outward, the middle with the convexity directed inward. At the point of junction of the anterior and middle sections, in the furrow $\frac{\text{XVIII}}{\text{XIX}}$, the groove enlarges into a small triangular sinus containing a minute papilla upon which the male pore opens. This is perhaps situated rather more on XIX than XVIII. At each end of the groove and just external to the closely approximated penial setæ of somites XVIII and XX are the external openings of the two pairs of prostate glands, the secretion of which is therefore brought to the sperm by means of this groove. The female pores are situated close together between the ventral setæ of XIV, but are invisible in surface views.

No complete cœlomic septa exist anterior to $\frac{\mathrm{V}}{\mathrm{VI}}$, this space being largely occupied by the radiating pharyngeal muscles; $\frac{\mathrm{XII}}{\mathrm{XIII}}$ is slightly thickened, $\frac{\mathrm{VI}}{\mathrm{VII}}$ and $\frac{\mathrm{XI}}{\mathrm{XII}}$ about twice as thick, and $\frac{\mathrm{VII}}{\mathrm{VIII}}$ to $\frac{\mathrm{X}}{\mathrm{XI}}$ each about four times as thick as ordinary septa and extremely muscular. From $\frac{\mathrm{VI}}{\mathrm{VII}}$ to $\frac{\mathrm{VIII}}{\mathrm{IX}}$ the septa are strongly funnel-shaped and "nested." The pharynx occupies III and IV, a short œsophagus V, the strongly muscular gizzards VI and VII; then follows a straight narrow canal which appears to lack calciferous pouches, but is slightly enlarged with yellowish, thickened and very vascular walls in the posterior part of X and presents a similar but less pronounced structure in XI. The true sacculated intestine begins in XVIII. The anus is a wide vertical slit. The brain is transverse, about five times as wide as long, without median constriction or any lobing, and is situated in II. The ventral cord is remarkable for the complete and extremely thick muscular sheath, which equals ½ the diameter of the cord proper at a ganglionic enlargement. Although present in other species of *Diplocardia*, the muscular sheath is in this one thicker than usual. Apparently there are no peculiarities of the vascular system. Unlike the type species the dorsal vessel is single and undivided throughout in the three examples dissected. Strongly enlarged hearts occur in XIII, and a slightly enlarged pair in XII. The nephridia begin in III, and appear to be present in every succeeding somite. Except for the first five pairs, which are more compactly coiled and of smaller size, they have the long-looped form described for other species of the genus.

Three pairs of spermathecæ exist in the anterior parts of somites VII, VIII and IX, attached to the body floor at the base of the corresponding septa, behind which they rise freely to a high level, though occasionally they pass through the neural arch of the septum into the preceding somite. All of the spermathecæ have the pouch and stalk very distinctly differentiated and are especially characterized by the great length of the latter and the very low position of the diverticulum which arises from its lateral side at the point at which it enters the body wall. The pouch has in general the form shown in fig. 1, which represents the second spermatheca, and in all of the specimens examined is strongly flattened laterally. The stalk is at least as long as and usually longer than the pouch, and also more or less flattened by the pressure of the

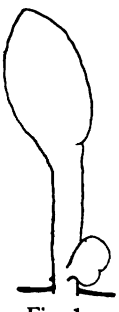
Fig. 1.

neighboring organs. The flat, spreading diverticulum opens into the base of the stalk by a short, narrow duct. In three specimens dissected the pouch increases in size successively from the first to the third spermatheca and the diverticulum diminishes correspondingly. While completely divided into two or even three lobes in the first, the latter is in the second and third either simple or very faintly bilobed.

The testes occupy the usual position in somites X and XI, and two pairs of sperm sacs are borne on the anterior face of septum $\frac{\text{IX}}{\text{X}}$ and the posterior face of septum $\frac{\text{XI}}{\text{XII}}$. The latter are much and deeply lobulated, having the aspect of dense tufts of broadly clavate or stalked spheroidal bodies. The sperm funnels lie in somites X and XI attached to the posterior septa near the cœlomic floor and close to the ventral nerve cord. They are of simple form and consist chiefly of a pair of broad lobes partly folded together like the two lobes of a *Dionæa* leaf. After penetrating the septa the vasa deferentia plunge at once into the longitudinal muscular layer of the body wall which they penetrate to a depth of about $\frac{1}{5}$ of its thickness, and continue at this level in the line of the ventral setæ to a point near the male gonopore, when they bend sharply outward toward the surface. Throughout their course the two vasa deferentia of each side lie side by side, but are perfectly distinct until quite at the external orifice, where they coalesce at a common opening. The ovaries are conspicuous, fimbriated, fan-shaped structures consisting of numerous chains of ova attached to the posterior face of septum $\frac{\text{XII}}{\text{XIII}}$. Trumpet-shaped oviducts, the mouths of which are formed very much like the sperm funnels, perforate the septum $\frac{\text{XIII}}{\text{XIV}}$, and at once penetrate the body floor obliquely backward and inward. Near the external surface they bend sharply toward the median line and open in the setæ zone on the ventral surface of XIV, separated by an interval of about $\frac{1}{4}$ *a-a* or less than the transverse diameter of the nerve cord with its sheath. The external pores are so small that they can be detected only in sections. The prostate glands (fig. 2) are large and conspicuous, and are variously folded sharply back and forth several times within the limits of the single segments XVIII

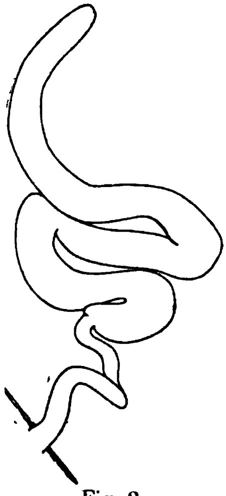
Fig. 2.

and XX which the two pairs respectively occupy. The glandular portion and the muscular duct are sharply differentiated, and the latter arises directly from the end of the former. The former is about three times as thick and three and one-half times as long as the latter, cylindrical and tubular, with a distinct lumen throughout and thick walls composed of gland cells in various stages of activity.

The integuments are translucent and the colors dull in life. The anterior and usually the extreme posterior ends are hair brown, varying in shade; the clitellum is russet, and the remainder of the body nearly salmon pink or sometimes ecru. Through the less pigmented portions of the skin the blood vessels show conspicuously of a deep purple color. These worms are very sluggish, but may be stimulated to greater activity by exposure to light, which they seek to avoid. They occur in moist sandy soil.

A NEW SPECIES OF SEA-MOUSE (APHRODITA HASTATA) FROM EASTERN MASSACHUSETTS.

BY J. PERCY MOORE.

The common European sea-mouse (*Aphrodita aculeata*) has been so frequently and so widely reported from the American Atlantic coast, while none of the writers on our annelids have reported any other species, that the identity of the species occurring on the two sides of the Atlantic has been taken as established. It was, therefore, with genuine surprise that I found, while preparing a description from Wood's Hole specimens for a report on the annelids of that region, certain obvious points of difference between these and *A. aculeata* as described by European writers. Since returning to Philadelphia a more thorough examination of the literature and a detailed comparison of specimens in the collection of this Academy with those belonging to the U. S. F. C. laboratory at Wood's Hole, kindly sent to me by Mr. Vinal Edwards, and two specimens of *A. aculeata* from the neighborhood of Helgoland, for the opportunity of studying which I am indebted to Dr. W. McM. Woodworth, of the Museum of Comparative Zoology, have doubly convinced me of the wide distinction between the species common in the deeper waters of the open sea off the Wood's Hole region and the European species. This does not, of course, exclude the possibility of the occurrence of the true *A. aculeata* also on our coast, yet the few notes furnished by Prof. Verrill in his *Report on the Invertebrates of Vineyard Sound* lead to the belief that the species therein recorded as *A. aculeata* is the one herein described, which it seems probable is the only one occurring south of Cape Cod.[1]

A. hastata is really less closely related to *A. aculeata* than to other species of the genus and probably finds its nearest ally in *A. japonica* Maren., which is widely distributed in the northern Pacific. From that species it differs in having the notopodial setæ free from the felt and in the decidedly smaller number of neuropodial setæ, which also lack the terminal pilosity in all of those examined.

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[1] Mr. James E. Benedict writes me that Prof. Verrill has informed him that two species of *Aphrodita* are found on our coasts. Owing to the illness of Miss Bush, to whom their discrimination is said to be due, I have been unable to secure further information.

From *A. aculeata* it departs in many and striking characters. Perhaps the most important is the altogether different form of the large notopodial spines. In the former they are acute, rigid needles whose points project stiffly a short distance above the felt, and are capable of inflicting quite painful wounds. In the latter they curve over the back to or beyond the middle line and are soft, flexible but friable, and terminate in acute and hooked tips. It is interesting to note that McIntosh states that the young of *A. aculeata* possess setæ of this sort, which are later replaced by the acute spines. But *A. hastata* and several other species retain the more primitive form throughout life, unless, of course, as frequently happens, they are accidentally injured. In *A. aculeata* the number of neuropodial setæ is constantly greater in the middle and ventral rows and sometimes greater in the dorsal row, and they are stouter, less acute and differ otherwise in form. The lateral fringe of hairs is brilliant green in *A. aculeata*, pearl color or reddish in *A. hastata*, and there are other minor differences. A dissection of a single example of *A. hastata* indicates identity in the internal anatomy of the two species. The complete description follows:

Aphrodita hastata sp. nov.

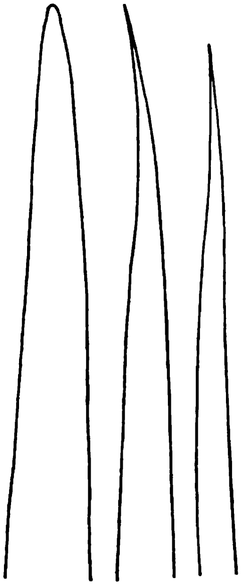
The size is large, examples of 125 mm. long and 40 mm. in maximum breadth at somite XII, exclusive of the setæ, being common, though none equalling the maximum size of *A. aculeata* has been seen. Examples of 70 to 125 mm. have 40 or 41 somites, the last 15 or so being very small and comprising not more than one-eighth of the total length.

As in *A. aculeata*, the form is robust and strongly arched in the anterior half, the last fourth becoming slender and tapering rapidly in both planes. The ventral surface is relatively smooth and pale, the brownish spherical papillæ being few in number but increasing toward the sides and becoming numerous on the dorsal, anterior and posterior surfaces of the parapodia. Hidden, of course, beneath the dorsal felt are 15 pairs of elytra and the dorsal fimbriated organs, both arranged as in *A. aculeata*.

The prostomium is orbicular obovate; the greatest width, which lies near the anterior border, is about equal to the length and the posterior half tapers rapidly to a width of about one-third the maximum. The two minute, closely approximated eyes on each side are placed just anterior to the greatest breadth of the prostomium and separated by a wide median interspace. Although apparently quite variable the facial tubercle is nearly always considerably shorter than the prostomium, strongly compressed below, broad and usually with a median groove above. While the usual spherical papillæ stud its surface it is otherwise smooth and exhibits little of the nodular character seen in some species. The median tentacle has a total length slightly exceeding the prostomium, of which about one-fourth or more constitutes the strongly clavate basal piece. The style is slender and regularly tapering, and in no case exhibits any trace of the club-shaped extremity figured by McIntosh for *A. aculeata*. The palpi are from six to eight times as long as the prostomium and nearly twice as long as the first parapodium and tentacular cirri; in form they are slender and regularly tapering.

Nothing peculiar appears in the form or modifications of the parapodia, which resemble those of *A. aculeata*, though the first pair appear to be rather longer, their tips reaching about 1½ times the length of the prostomium beyond the latter. The ventral cirri reach to the middle row of neuropodial setæ, while the dorsal cirri extend fully one-third of their length beyond the tips of the longest of the latter, being therefore relatively somewhat longer than in *A. aculeata*.

The general arrangement of the several forms of setæ is that usual in the genus. Of the three series of neuropodials the dorsal invariably contains two, the middle usually 4, and the ventral 8, though 5 may occasionally occur in the middle and 7 to 8 in the ventral series. All of these setæ are brown and decidedly iridescent, and the slender ones quite pale. In the dorsal row both spines (fig. 1) are very stout, and both terminate in blunt points which probably result from wear, though not a single one of these spines in any of my specimens presented a really acute tip. The middle setæ (fig. 2) are moderately and the ventral (fig. 3) decidedly slender, and both are similarly formed, with acute attenuated tips not preceded by any enlargement and not concealed in a pilose coat, though those newly extruded are sheathed. In the dorsal series the anterior seta is frequently stouter, while those in the other two increase in size from before backward. Toward the caudal end, coincidentally with the reduction in size of the parapodia, these setæ become more slender, smaller and fewer, and on the last 7 or 8 parapodia are altogether wanting.



Figs. 1, 2 and 3.—Neuropodial setæ from somite X. Anterior of dorsal, posterior of middle, and middle of ventral series, respectively. All × 56.

From the lower side of the notopodial tubercle arises a tuft of iridescent hairs which spring from a curved line reaching from the anterior margin of the tubercle upward and backward to the base of the notopodial cirrus, which occupies a posterior position. These capillary setæ spread in a flowing plume outward, backward and upward, covering the sides of the body and the neuropodia. Compared with the corresponding structures in *A. aculeata* they present numerous differences. In the first place they are fewer, about one-third longer and spread more widely, irregularly and in greater disorder from the sides. In our species they are much softer and less harsh and rigid—a difference which becomes especially evident if they are brushed forward and released, when those of *A. aculeata* spring quickly back to position, while those of *A. hastata* return slowy and gently. But the most striking difference is in color, ours being far less brilliant than the European species. When placed in corresponding positions with reference to the light and the observer's eye, namely, with the caudal end toward the light and the head toward and below the observer, the marginal hairs of *A. aculeata* appear of a beautiful burnished golden green color, rich golden predominating toward the base and a fine viridian green in the outer half, the intensity of the display being enhanced by the great number and relatively compact arrangement of the hairs, while the general effect of those of *A. hastata* varies from a pearl color to a richer bronzy red in different individuals. In the paler variety the hairs appear purplish-blue, toward the base changing to a delicate red, and toward the tip to a varied mixture of reddish-purple and bluish-green which differs as the glancing light strikes particular hairs at different angles. Owing to the more open, spreading arrangement of the hairs in our species this dispersal of the colors is more evident. When viewed at other angles or by transmitted light the distribution of colors differs.

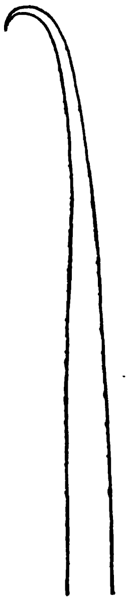


Fig. 4.—Tip of notopodial seta. × 250.

The two tufts of large notopodial spines arise, as in most species, one just anterior to the dorsal cirrus, the other, separated by a short interval, higher on the dorsum. The first consists of about 4 and the second of about 6 or 7 long, curved, soft, coarse and brownish setæ which, after perforating the dorsal felt, curve, most of them perfectly free from and above the latter, caudad and mediad, often crossing those of the opposite side and many of them attaining a length in excess of the greatest width of the body. At the base they are very coarse, but taper gradually to the tip, which is recurved as an acutely pointed hook (fig. 4). In section they are often flattened and seldom perfectly circular. The interior consists of a core of soft colorless fibres which are enclosed in a firmer and brittle sheath or shell of a more or less iridescent brown color. It is to this structure that these spines owe their softness and fragileness, the latter quality being so marked that large specimens almost invariably have all of them broken short off above the felt, leaving the latter exposed over the entire median expanse of the back, and giving to this species an aspect which has naturally led to its identification with *A. aculeata*.

The felt fibres arise in three tufts, one ventral, one between and one dorsal to the dorsal setæ bundles. In large specimens the felt forms a uniform continuous layer nearly ⅛ in. thick and of a smooth, compact texture. The fibres appear to be finer than in the two specimens of *A. aculeata* available for comparison. They also have less color, many of them being altogether dull and colorless while others exhibit a slight greenish iridescence.

The type is No. 20, Collection Acad. Nat. Sci., and was taken by Dr. Benjamin Sharp on the beach at Nantucket after a storm. About a dozen other specimens have been examined, coming partly from the same place, partly from the collections of Mr. Vinal Edwards on Noman's Land and from dredgings of the U. S. F. C. steamer *Fish Hawk* in the deeper waters off the same region. The species has not been taken in the course of the recent extensive dredgings of the *Fish Hawk* and *Phalarope*, either in Vineyard Sound, Nantucket Sound, or Buzzard's Bay. Occasionally, it is brought up in lobster pots set in the deeper waters off Noman's Land, and it is probably this species which is said to be sometimes thrown up in great numbers during heavy storms on the shores of Block Island.

SOME MARINE OLIGOCHÆTA OF NEW ENGLAND.

BY J. PERCY MOORE.

That the study of the littoral Oligochæta of the eastern United States has been much neglected becomes evident when it is mentioned that but four original references to the subject occur in the literature, and three of these are very brief diagnoses of species. The first is found in Prof. Leidy's *Contributions to the Marine Invertebrate Fauna of Rhode Island and New Jersey*, published in 1855, where *Lumbriculus tenuis* is described from specimens taken at Point Judith, Rhode Island. In 1863, Minor gave a quite recognizable description in the *American Journal of Arts and Sciences* of his *Enchytræus triventralopectinatus*, taken near high-water mark at New Haven, Connecticut. In 1873, in the *Report upon the Invertebrate Animals of Vineyard Sound*, Prof. Verrill describes as new *Clitellio irrorata* and *Halodrillus* (gen. nov.) *littoralis*. The first three of these have not since been reported by any other observers, but the last was carefully studied and its anatomy described in a *Bulletin of the Illinois State Laboratory of Natural History* for 1895 by Smith, who establishes its identity with a widely distributed species of *Enchytræus*. Michaelsen, in his *Monograph of the Oligochæta*, recognizes the close resemblance between Minor's species and the well-known *Paranais littoralis* of Europe. The remaining two species have been noticed repeatedly by monographers and systematic writers who have been unable to arrive at any satisfactory conclusions as to their status.

While engaged in studying *Polychæta*, chiefly in the region about Wood's Hole, Massachusetts, but also at other points on the New England coast, for several years past, the writer has collected a number of species of littoral *Oligochæta* which, besides permitting the identification of the previously recorded species with certainty, except in the case of *Lumbriculus tenuis*, embrace several interesting additions to our known fauna. Minor's *Enchytræus triventralopectinatus* proves to be *Paranais littoralis*, as supposed by Michaelsen; *Lumbriculus tenuis* Leidy is almost certainly *Clitellio arenarius* (Müller); *Halodrillus littoralis* Verrill is *Enchytræus albidus* Henle; and *Critellio irrorata* Verrill is a composite of *Tubifex irroratus* (Verrill), *Tubifex benedeni* Udekem and

Clitellio arenarius Müller. To the latter conclusion I have been much assisted by Prof. Verrill's great kindness in sending to me for study his entire collection of marine oligochætes, including many bottles labelled by him as *Clitellio irrorata.* Although this material was collected from 25 to 30 years ago, and had since been subjected to all of the vicissitudes of preservation in small vials of alcohol, it was possible to determine most of the specimens, and as a result to considerably extend the known range along our coasts of several species. For this privilege I am much indebted to Prof. Verrill.

In the present paper are noticed ten species representing three families, as follows: Naiidæ, *Paranais littoralis;* Tubificidæ, *Clitellio arenarius*, *Monopylephorus glaber*, *M. parvus*, *Tubifex irroratus*, *T. benedeni*, *T. hamatus* and *Limnodrilus subsalsus;* Enchytræidæ, *Enchytræus albidus* and *Lumbricillus agilis.* Of these *P. littoralis*, *C. arenarius*, *M. parvus*, *T. benedeni* and *E. albidus* are European species, but only the first and last have hitherto been recognized on our shores. *Tubifex irroratus* is the residuum of Verrill's *C. irrorata* and the remaining four are new, but have more or less closely related representatives in other parts of the world. It needs to be remarked that very few of these worms are really marine; they are more or less recent migrants from a rich limicoline or terrestrial oligochæte fauna. The naids and tubificids are derivatives of the former which have been carried down the streams into bays and estuaries and have spread through the brackish waters and along the littoral zone. In their new habitat they have undergone modifications that are sometimes considered to have generic value. A case of especial interest is that of the brackish water *Limnodrilrus subsalsus*, which is the only species of this large genus that has been recorded as occurring out of fresh water. As pointed out in the systematic part of the paper, especially under *Monopylephorus glaber*, some of the tubificids burrow in soil saturated with fresh water from springs, and are experimentally shown to take more kindly to pure fresh water than to salt water of sea density. The enchytræids are more particularly strand forms which inhabit the marine vegetation thrown by the waves upon the beach. They enter the littoral zone, but are often found in large numbers quite above the reach of the waves at high tide, particularly during rain storms. They are undoubtedly derived from terrestrial or amphibious forms that have found a favorable environment on the seashore. *Enchytræus albidus*, at least, is found in garden and wood soil also, but may have been introduced through the use of eel grass for fertilizer.

The species of Tubificidæ described in this paper, as well as some of those described recently by other authors, tend to traverse some of the generic boundaries usually set up and to obscure their limits. *Tubifex* is here employed in a broad sense, and it will be noticed that *T. irroratus* approaches *Monopylephorus* in many respects and removes that genus from the isolated position that it has been considered to occupy. This species resembles *Monopylephorus* in its valvular and moniliform blood-vessels, the form of its nephridia and the short sperm ducts with diffuse prostate glands. On the other hand, the paired genital orifices, the well-developed penes and the capillary setæ in the dorsal bundles are characters of *Tubifex*. Ditlevsen has referred to *Monopylephorus* a species (*M. trichochætus*) having all of these characters except the penes. The supposed great gap between these genera is thus bridged by two species. On the other hand, *Tubifex hamatus*, which is in most respects a typical *Tubifex* of the *Psammoryctes* group, appears to lack capillary setæ altogether. Some of our forms here referred to European species exhibit slight departures from the published descriptions, and it is possible may prove to be distinct when actual comparison of specimens from both places comes to be made. Besides the species described in this paper my collections include several others represented only by immature specimens, from which the characters of the reproductive organs cannot be ascertained.

The following key will facilitate the determination of species noticed in this paper:

A.—Asexual reproduction by serial budding and fission.
  B.—Spermathecæ in V; setæ all hooked and bifid, the dorsal bundles beginning on V; size very small, *Paranais littoralis*.
AA.—Reproduction normally by the sexual method only.
  BB.—Spermathecæ in V; setæ all with simple (not bifid) tips and dorsal bundles beginning with the ventral on II; male pores on XII.
    C.—Setæ *f*-shaped; blood yellowish; testes deeply lobulated, *Lumbricillus agilis*.
    CC.—Setæ straight, with hooked internal ends; blood colorless; testes not subdivided, . *Enchytræus albidus*.
  BBB.—Spermathecæ in X; setæ more or less distinctly bifid at the tips and of various forms, often associated in the dorsal bundles with capillary setæ; male genital pores on XI.
    D.—Male genital pores and spermathecal pores paired; penes present.
      E.—Atrium simple; no special prostate gland; spermathecæ reaching into XI when fully developed, ... *Clitellio arenarius*.

EE.—Atrium divided into penis sac and sperm reservoir, the latter with a special prostate gland; spermathecæ confined to X.

F.—Sperm ducts long and slender; prostate glands massive.

G.—Cuticle more or less completely covered with flattened papillæ; capillary setæ present or absent; no copulatory setæ, *Tubifex benedeni.*

GG.—Cuticle without papillæ; capillary setæ always(?) absent; posterior dorsal setæ enlarged and strongly hooked; copulatory setæ present on X, ... *Tubifex hamatus.*

GGG.—Neither cuticular papillæ, capillary setæ, nor copulatory setæ present; vas deferens very long and penis sac very complex, with spirally wound muscular coats and a trumpet-shaped chitinoid penis sheath, *Limnodrilus subsalsus.*

FF.—Sperm ducts short and wide; prostate glands diffuse.

H.—Penes present; genital pores paired; capillary setæ present, *Tubifex irroratus.*

DD.—No penes nor capillary setæ; spermiductal and spermathecal pores median or nearly so; sperm ducts short and wide; prostate glands diffuse.

I.—Spermathecæ paired; setæ all distinctly bifid at tips, *Monopylephorus glaber.*

II.—Spermatheca single; bifurcation of setæ often obsolete, *Monopylephorus parvus.*

Paranais littoralis (Oersted) Czerniavsky.

Nais littoralis Oersted, 1843.
Enchytræus triventralopectinatus Minor, 1863.
Paranais littoralis Czerniavsky, 1880.

This species was described under the name of *Enchytræus triventralopectinatus* as long ago as 1863, from New Haven, Connecticut, by Minor, but appears to have escaped subsequent notice in America. Michaelsen places Minor's species doubtfully among the synonyms of *P. littoralis.* Hitherto budding individuals only have been found, and until the characters of the mature reproductive organs of American examples are known the identity of the latter with the European species cannot be considered as established. Nevertheless, so far as budding worms are concerned, no distinctions can be detected, unless the number of setæ averages somewhat less in American specimens. The prostomium is often slightly pigmented, but there are no eyes. The blood is pale greenish-yellow, and the chlorogogue cells pale brown. The first budding zone usually occurs at XX or between XIX and XX. A second may occur at XXXV, but usually only the first is conspicuously developed. Still attached zooids may possess as many as 28 or 30 segments before the appearance of a second budding zone. In buds nearly ready to become detached both dorsal and ventral setæ occur on all segments. All through July and August active budding continues under natural conditions, but worms kept throughout the autumn at Philadelphia failed to develop genital organs.

Nothing but its small size and mode of occurrence has caused this species to be overlooked for so many years as it is extremely abundant and widely distributed. More than any other species it withstands a wide range of density in the water, being almost equally at home in the rain-soaked eel-grass above high tide, on the shores of brackish ponds and under stones near low-water mark on the exposed shores of Vineyard Sound. Its movements are characterized by frequent quick, nervous turnings and contractions.

Clitellio arenarius (Müller) Savigny.

Lumbricus arenarius Müller, 1776.
Clitellio arenarius Savigny, 1820.
?*Lumbriculus tenuis* Leidy, 1855.
Clitellio irrorata Verrill (in part), 1873.

Of the ten species noticed in this paper the present one is the largest, full-grown examples exceeding two inches in length and a millimeter in diameter at the clitellum. Preserved specimens usually have the mouths of the spermathecæ and the penes protruding in the form of prominent papillæ, as Prof. Verrill has noted in his description. Sexually mature worms are abundant, enabling one to secure cocoons in numbers and to study the anatomy of the genital organs with ease. There is little doubt of the identity of the American and European worms, the only noticeable differences being a slightly greater average number of setæ in ours and an apparently somewhat shorter spermathecal duct than is figured by European authors. No entirely satisfactory figures of the setæ of the European worm have been found, and it is possible that there may be a difference in their form also. The spermathecæ are often filled with large vermiform spermatophores.

The distribution of this form is equally extensive on the European and American sides of the Atlantic. It is excessively abundant in

Casco Bay and northward in Maine, where it occurs under stones and among grass roots up to high-water mark on muddy and gravelly shores. While apparently less plentiful south of Cape Cod, it is common at many suitable points on the shores of Vineyard Sound, Buzzard's Bay, Narragansett Bay and Long Island Sound.

Without being able to decide the matter definitely, it seems extremely probable that this is the species described by Leidy. Although the characters given in the brief description of *L. tenuis* are not diagnostic, they are sufficient to eliminate every other species described in this paper except the brackish water *Limnodrilus subsalsus*, while they all and the character of the habitat, as well, apply to *C. arenarius*. Vaillant, indeed, has already referred *Lumbriculus tenuis* doubtfully to *Clitellio*. Later writers have been more cautious.

In Prof. Verrill's collections this species occurs far more frequently than any other and usually under the name of *Clitellio irrorata*, thus giving the best of authority for the inclusion of the latter in the synonymy. It is this species that is figured under the name of *C. irrorata* in *Trans. Conn. Acad.*, 1881, Pl. VIII, figs. 3 and 3*a*. The localities represented cover a wide extent of coast and, named from north southward, are as follows: Nova Scotia, Bay of Fundy, Casco Bay, Cape Ann, Gloucester, Massachusetts; Salem, Massachusetts; Cape Cod, Vineyard Sound, Wood's Hole and New Haven.

Monopylephorus glaber sp. nov. Plate XXXII, figs. 1–6.

This species is relatively short and stout for a tubificid, the largest examples not exceeding 40 mm., and the usual length of living mature worms being about 25–30 mm., with a diameter of .8 mm. Young examples are relatively much more slender. In mature worms there are from 67–74 segments, and generally they are 4-ringed, bearing the setæ on the third and consequently well behind the middle. The greatest diameter is at the clitellum and genital region, from which the body tapers rapidly to the small pointed prostomium and very gently to the anal somite. The clitellum occupies part of X, all of XI and XII, and sometimes extends on to XIII. Except for the sensory hairs belonging to a zone of cutaneous organs encircling the middle of each somite, the skin is devoid of cuticular pilosities and perfectly smooth. Owing in part to the presence of grayish-brown pigment granules, and in part to the remarkably high peritoneal layer of cells, the body walls are decidedly opaque. The external opening of the genital bursa is a conspicuous median, transversely elongated aperture in the setæ zone on the venter of XI. The female pores are paired in the furrow

$\frac{\text{XI}}{\text{XII}}$, and the spermathecæ open close together in a common depression just behind furrow $\frac{\text{IX}}{\text{X}}$.

The general color varies from pinkish in young worms to brown or purple in fully grown ones, in which the color is due to a combination of the rose of the blood with the brown of the chlorogogue cells, the opaque white of the cœlomic corpuscles, the dark contents of the intestine and the pigment of the skin. Anteriorly the color remains pink or, through the accumulation of cœlomic corpuscles, becomes opaque white. When, as in certain individuals, the latter are very numerous, they impart a characteristic whiteness and opacity to the entire worm.

The setæ are all of the usual short, bifid, hooked form (fig. 4), with a deeply cleft tip and the terminal process somewhat longer and more slender than the widely divergent basal one. The portion of the seta external to the body wall is peculiarly thickened, but otherwise presents no characteristic features. In each bundle the inner setæ are somewhat the longer and those of ventral bundles slightly exceed the dorsal, but no marked differences are anywhere noticeable. As usual, the ventral bundles of XI are wanting. From II to XI 4 or 5 is the prevalent number in each bundle, the last being most usual in the ventral bundles, though they occasionally contain 6. Behind the clitellum 3 is usual in the ventral, 2 in dorsal bundles, and both may become reduced to one toward the posterior end.

Owing to the opacity of the body walls and the profusion of cœlomic corpuscles in mature worms the internal anatomy is difficult to work out upon living subjects, though practically all of the results of the study of sections have been confirmed upon living material and dissections. The spherical cœlomic corpuscles (fig. 5) are from .009 mm. to .0125 mm. in diameter, and filled with a comparatively small number of spherical granules measuring .0025 in diameter, and whose opacity and whiteness gives to the corpuscles their characteristic aspect. Among these corpuscles are seen a few filled with very much smaller granules which appear to be budded from the high peritoneal cells.

No unusual features are presented by the alimentary canal. The pharyngeal region is very small, though its glands extend diffusely to V or VI; the simple intestine is regularly and prominently sacculated, especially behind the intestine, and except in the genital region, where they are slightly developed, and in somites IV and V, in which they make their appearance, is thickly enveloped in chlorogogue cells, which largely conceal the dorsal vessel also.

As in all members of the genus hitherto described, the vascular system presents a well-developed integumental plexus on the lateral arches and a remarkable system of unicellular valves. The ventral vessel is free from the intestine, without sacculations and without valves. It bifurcates in III, but is usually reunited in II by an asymmetrical commissural vessel, from which a small azygous vessel arises. The dorsal vessel is contractile, sacculated and provided with a set of valves at each constriction throughout its entire length as far forward as somite III. Although its anterior end is largely free from the intestine, it retains a covering of chlorogogue cells as far as somite IV and bifurcates only on reaching II. A pair of small lateral arches without valves arises in I, and after dividing each joins the here double ventral vessel. Beginning with III, the arches spring from the dorsal trunk at the posterior ends of the somites just anterior to the valvular constrictions. They reach to the body walls, which they enter and then divide into a complex integumental network emptying into the ventral vessel by two trunks in each somite, while the plexuses of contiguous somites are united by a longitudinal connecting vessel. The network is similar to that of *M. pilosus*, but appears to be somewhat more open. The lateral contractile trunks increase in size to X, one of which loops into the sperm sac, while one in XI forms a rich plexus within the ovisac. Behind XI they become much smaller, but continue to form integumental plexuses which join the ventral vessel by two trunks. The arches from III to XI are moniliform and provided with valves at each constriction. That in III contains one set of valves, IV 2 sets, V 2 or 3 sets, VI and VII 3 or 4 sets, and in VIII to XI 5 or 6 sets—numbers which, while occasionally varying, are remarkably constant. In the larger arches the two primary branches also may each contain a set of valves.

The brain (fig. 3) has a peculiarly lobulated surface and is broad and very deeply incised anteriorly, somewhat narrower and scarcely emarginated posteriorly, where the muscular sheath is produced into a pair of contractile strands, between which the dorsal vessel passes. Ordinarily the brain is about ½ longer than broad, but the proportions vary greatly with the state of contraction and extension of the worm.

Nephridia (fig. 2) begin in VII with their nephrostomes opening into VI; none is present in X and XI, though a pair communicating with the latter is frequently developed in XII. In young worms, in which the transparent body wall permits the nephridia to be readily studied, they are absent from many somites and developed on one side only of others, but in full-grown worms few segments, except at the posterior region, lack them. The funnel is small, with the prolonged lip or tongue more slender and elongated than in *M. pilosus*. It passes into a short neck, which penetrates the septum and joins an irregular massive region in which the canal is much folded, with granular coalesced walls and irregular lumen in the anterior part, while in the posterior part the lumen becomes more regular and provided at intervals with ciliated ampullæ. From this portion an elongated lobe passes caudally and medially along the ventral blood-vessel. In this lobe are four canals arranged in two loops, the proximal one of which contains ciliated ampullæ, while the distal one lacks cilia and, after returning to the massive region, passes into a large thick-walled efferent canal, the lumen of which exhibits a few irregular lateral diverticula, and which finally opens to the exterior by a pore situated a short distance anterior to the ventral setæ bundles.

The reproductive organs agree closely with what is known of other members of the genus. A pair of testes suspended from the posterior surface of the septum $\frac{\mathrm{IX}}{\mathrm{X}}$ fill not only the cœlome of X, but large anterior and posterior sperm sacs. The former is a short, wide, nearly spherical diverticulum from the septum $\frac{\mathrm{IX}}{\mathrm{X}}$ which arises dorsal to the intestine and projects forward when fully developed to the septum $\frac{\mathrm{VIII}}{\mathrm{XI}}$. The posterior sac is much longer and, arising from the septum $\frac{\mathrm{X}}{\mathrm{XI}}$ above the intestine, accompanies, but usually does not enter, the ovisac to about somite XIII or XIV. In young worms there are two, but later one disappears. Before maturity the cavity of the functional one is closed by a transverse diaphragm which prevents the vascular arch from entering more than a short distance.

The large sperm funnels rest on the floor of X against the posterior septum, which is perforated on each side of the nerve cord by the short vasa deferentia, which then bend laterally and each expands into a slightly enlarged region covered completely by a continuous layer of rather small prostate gland cells. This region—the sperm reservoir—bends upward around the intestine, and at the highest point passes into a short ductus ejaculatorius free from glands and opening into the summit of the fusiform penis sac. The latter is provided with a very deep epithelium thrown into rugous folds and relatively thin muscular layers; but there is no trace of a penis. The atria of the two sides open into the lateral portions of the summit of an eversible median bursa, which probably acts to some extent as a substitute for the penis (fig. 1).

Ovaries occupy a position in XI relatively similar to that of the testes in X, and are provided with a median dorsal ovisac which, arising from the septum $\frac{\mathrm{XI}}{\mathrm{XII}}$ above the intestine, reaches to XVI or beyond. In addition to the ova it receives a vascular network, usually from the left arch of the pair in XI, and sometimes the posterior sperm sac in addition.

Spermathecæ (fig. 6) are nearly always conspicuous even in young worms. They open either very close together or by a common median pore just behind the furrow $\frac{\text{IX}}{\text{X}}$, and consequently are well forward in the latter somite. They have short, thick, muscular ducts without glands, and ovoid or sometimes nearly spherical ampullæ which when fully distended may reach nearly the length of the somite. When fully distended the lining epithelium is thin and flat and the muscular layer thin, but when nearly empty the former becomes thick and folded, often in a spiral or annular fashion, a condition which usually prevails near the entrance to the duct, where the circular muscular layer is much thickened. The spermatozoa are never fashioned into spermatophores, but fill the ampulla in compact masses and bundles.

This species is closely related to *Monopylephorus rubronivens* Levinsen, to which Ditlevsen has recently referred *Vermiculus pilosus* Goodrich. From this species, as elaborately described by Goodrich, *M. glaber* differs in the entire absence of villous processes on the cuticle, the greater number of setæ, the form of the nephridia and some minor characters of the vascular system, brain, etc.

Another closely related species is *M. fluviatilis* (Ferronnière), but if the sperm ducts are correctly figured for the latter the position of the prostate glands is quite different.

Both Goodrich and Ditlevsen have attached much importance to the form of the nephridia, which they consider to approach the enchytræid type. In *M. glaber* this resemblance is entirely superficial and the arrangement of the tubules is nearly typically tubiciform, their analysis into glandular region, large and small tubule loops being easy.

In suitable localities this species is very abundant about the shores of Vineyard Sound and Buzzard's Bay. It prefers regions overlaid by a layer of fine organic mud in places where there is an accumulation of decaying eel grass or other vegetable matter, and especially where the salt water is diluted by springs or streams of fresh water. In such places it occurs in enormous numbers beneath stones or a short distance below the surface, while in nearby spots, where the water is of much greater density but the conditions otherwise similar, few or none will be found. Nowhere in this region have these worms been found in greater numbers or of such large size (mostly exceeding 40 mm. in length and 1 mm. in diameter) as along the gravelly and muddy banks of the Wareham river, a small stream emptying into the head of Buzzard's Bay. Here under stones near high water-mark, at a point where saltness of the water is just barely discernible to the taste, enormous numbers occur quite unmixed with other species. If placed in pure salt water of the density found in Vineyard Sound these worms die much sooner than if placed in fresh water, but may be acclimatized to either by gradual changes. In brackish water they have lived and bred in confinement in Philadelphia for seven months, and are now, after this lapse of time, as vigorous as ever. That they are partial to low rather than high densities is shown by the observation that those living on the shores of the Eel Pond at Wood's Hole, at a point where fresh water oozes from the ground below high-water mark, burrow deeper toward the source of the spring when the tide rises and covers their habitation, and come to the surface when it falls. Other favored localities are certain shallow and nearly closed coves where great quantities of eel grass and algæ accumulate and decay in water diluted by rains. Here under small stones below half-tide mark the species associates with *Tubifex irroratus* and *Lumbricillus agilis*, though the latter is far more plentiful in the decaying eel grass at a higher station on the shore, and the former prefers the roots of salt grasses growing on more gravelly shores. Though not active in their movements, they are less sluggish than the *T. irroratus* and far more hardy than that species. From the latter part of July, at least, onward this species breeds, and some specimens brought to Philadelphia continued sexually active throughout the fall and into winter. When breeding they are especially active and congregate in such numbers beneath stones that they impart a quite red color to the surface of the soil when exposed by turning over the stones.

Monopylephorus parvus Ditlevsen. Pl. XXXIII, figs. 29–34.

A second small species of *Monopylephorus* is referred to the above species, with which it agrees closely in nearly all of the characters recorded in the original brief diagnosis. Further information may necessitate a separation.

In size and form this species closely resembles *Lumbricillus agilis*, but its distinctly pink color and the opacity due to cœlomic corpuscles, as well as its more sluggish movements, are a ready means of separation. Fixed specimens can be distinguished in many cases only after a careful study, especially as sexually mature individuals are infrequent.

The length is from 8 to 15 mm., the diameter about .4 mm. and the number of segments from 38 to 43. The prostomium is much more slender and pointed than in *M. glaber*, but the somites are similarly quadri-annulate. The number of setæ in each ventral bundle is 3 or 4 anterior to the genital region and 2 posteriorly; in the dorsal bundles usually 5 anteriorly and 2 to 4 posteriorly. The setæ are all small and of the usual form, except that their tips are curiously variable (fig. 29). As the setæ were studied only under the rather unfavorable conditions presented by alcoholic material, it is uncertain how far this may be due to wear. Many of the setæ are undoubtedly broken, but those figured appear to be intact. In some the tips are deeply bifid and the points long and acute; others, especially in the posterior dorsal bundles, have the upper or distal point more or less reduced, and still others have a mere apical notch or are apparently entire.

The cœlomic corpuscles are very numerous and about .012 mm. in diameter, with opaque white granules smaller and more numerous than those of *M. glaber*.

In internal anatomy this species closely resembles *M. glaber*, from which, however, it is easily distinguished by the single spermatheca (fig. 34) which opens in the median line of the furrow $\frac{\text{IX}}{\text{X}}$, and is usually folded on itself transversely. The atria (figs. 32, 33) also differ in that they join in the middle line before opening into the bursa by a single small pore raised on the summit of a papilla. It will thus be noticed that the primitively strictly paired character of the reproductive organs has been departed from more widely in this than the other species. The prostate and other regions of the male ducts are otherwise as usual. An anterior sperm sac extends through IX, a posterior through XII and XIII, and an ovisac to XVI. The vascular system presents the characteristic valvular and chambered vessels and integumental plexus. The brain (fig. 31) is slender and the anterior lobes much elongated. Nephridia are of the character belonging to the genus, but are slender and elongated and the nephrostomial lip especially narrow and prolonged.

This species has not been found abundantly anywhere. Occasional examples occur with the larger tubificids and with *Lumbricillus*, but it appears to prefer more gravelly shores and the neighborhood of beach grass, among the roots of which it may be found. In a few cases larger numbers were found living gregariously beneath stones at half-tide on the south shore of Naushon. It is quite probable that it has often been mistaken for the young of *M. glaber* or even of *Lumbricillus*.

Tubifex irroratus (Verrill). Pl. XXXII, figs. 7–11.

Clitellio irrorata Verrill (in part), 1873.

The length of this slender species seldom exceeds 30 mm., but the number of somites may equal 90. Most of the examples found in the vicinity of Wood's Hole are not fully mature, and are from 15 to 17 mm. long, with about 70 somites. The prostomium is more acute than in *Monopylephorus*, and the somites are more elongated but similarly ringed. In mature examples the clitellum is developed on X, XI, XII and for a short distance on XIII. A pair of spermathecal pores occurs far forward on X just behind the furrow $\frac{\text{IX}}{\text{X}}$, and in line with the ventral setæ, the male pores occupy the place of the ventral setæ or a little behind on XI, and the ovipores are minute slits in the furrow $\frac{\text{XI}}{\text{XII}}$.

In young worms the color is pinkish, but in larger ones becomes deep brownish or purplish-red, often very dark, and in mature worms always very much darker than in *Monopylephorus glaber*, a difference which is probably due chiefly to the fact that the deep color of the chlorogogue cells is untempered by the whiteness imparted by great numbers of peritoneal corpuscles.

The setæ are of two forms, one kind being hooked and bifid and the other capillary and of very characteristic form. Both occur in the dorsal bundles. In preclitellar somites there are usually 2 or 3 or rarely even 4 of the former, with 1 or 2 or occasionally 3 of the latter alternating with, or 1 on the ventral side of each of, the hooked ones. Bundles of the middle region contain 2 hooked and 1 capillary seta, and those of the posterior region 1 of each. Normally every dorsal bundle except a few at the posterior end contains capillary setæ, but they are easily detached, and in some specimens fully formed ones are absent from as many as one-half or more of the bundles. When more than one is present they are usually of unequal lengths, the longest much exceeding the diameter of the body. They are very slender and taper regularly to the end, toward which they exhibit 2 or 3 very slight but distinct spiral turns (fig. 11). Possibly this region may be slightly flattened, though this appearance may be due to the lights and shadows caused by the spiral turns. The short hooked setæ (fig. 9) are always bifid, with the distal process decidedly longer unless worn or broken, which is seldom the case. Ventral bundles contain hooked setæ only which are quite similar in form to those of the dorsal bundles but somewhat larger; those of anterior bundles of both rows are larger than the posterior. Anteriorly the ventral bundles usually contain 3, in the middle region 2, and posteriorly 1. As usual the ventral bundles are wanting on XI, but those of X are not modified as copulatory setæ.

The vascular system of this species is remarkably like that of *Monopylephorus glaber*, presenting a similar valvular dorsal vessel, chambered and valvular lateral trunks, complex integumental plexus, and similar mode of branching. As distinctive characters it presents a finer and more numerously branched integumental plexus, with a more conspicuous longitudinal intersegmented trunk, and a greater number of sets of valves in the lateral arches. Usually the arches in III and IV have 1 set, V 1 or 2 sets ,VI 3 or 4, VII 4, VIII 4 or 5, IX and X 6 or 7, and XI 6. The right arch in X sends a loop into the sperm sac as far as the diaphragm present in the immature worm, while that of XI enters the ovisac and breaks up into a rich plexus. The intestinal plexus reaches as far forward as VII.

Owing chiefly to the height of the cœlomic endothelium the body walls are thick and opaque. Peritoneal corpuscles (fig. 10) are of two forms, about 90 per cent. of them being spherical and filled with rounded opaque granules having nearly the color of the vertebrate red blood corpuscle. The corpuscles measure .01 to .015 mm., and the granules .0012 mm. in diameter. The corpuscles are much less abundant than those of *Monopylephorus glaber*, and are easily distinguished by their smaller and more numerous granules. With the spherical corpuscles are associated a number of nearly homogeneous, colorless, flattened, fusiform corpuscles and a few leucocytes.

The brain is about as broad as long, very massive and with a slight median emargination posteriorly, and prolonged anteriorly into a pair of relatively slender lobes separated by a deep cleft.

Like so many other organs the nephridia have much in common with those of *Monopylephorus glaber*. The funnels are provided with a tongue which, however, is short and broad, and the remainder of the funnel is much lobulated. Nearly sessile on the septum, it passes into a short and narrow postseptal neck which, in turn, enters a large tubule with a very wide irregular lumen and highly granular walls which is doubled on itself and forms, with the first section of the tubule loops, the so-called glandular portion of the nephridium. The tubule loops have ciliated ampullæ and are in general arranged as in *M. glaber*, but their folds are much more open and in the posterior nephridia reach through two somites. The efferent canal springs from the glandular mass and opens to the exterior in front of the ventral setæ. Altogether the nephridium is of the true tubificid type. They are frequently developed on one side only or altogether absent from many somites.

The spermathecæ (fig. 8) are large, with prominent simple ellipsoidal ampullæ, varying much in size and shape with the degree of distension, but usually filling a large part of the cœlome of X, and reaching to the dorsal level of the intestine and the septum $\frac{X}{XI}$. There is always a well differentiated muscular duct without glands, short in distended spermathecæ, but relatively long in empty ones. There are no spermatophores, the spermatozoa being free and loose.

The male organs (fig. 7) are remarkable as combining the short sperm duct and diffuse prostate gland of *Monopylephorus* with the soft penis having a thin cuticular sheath of a typical *Tubifex*. Large funnels open into X on each side near the body floor and, after perforating the septum in line with the ventral setæ, pass into short wide vasa deferentia which bend laterally and gradually expand into the long sperm reservoirs constituting at least one-half of the entire duct. Near the posterior end of the somite they bend abruptly upward to a point above the intestine, and, by a short constricted region, pass into the summit of the penis sac, which is nearly vertical but curves forward to the external pores. The penis sac is cylindrical and has a thick muscular coat; the free penis begins at about its middle, but is usually considerably folded within the lower part of its cavity. There is a distinct but thin cuticular lining reflected on to the penis. The prostate gland is a thick layer of cells, especially enlarged on the dorsal side, uniformly covering the entire sperm reservoir.

The sperm sacs and ova sacs are quite similar to those of *Monopylephorus glaber*. When fully developed the anterior sperm sac reaches through IX, the posterior to XV and the ova sac to XXII. The posterior sperm sacs are originally paired and in immature worms may be seen to be swept back and forth, alternately reversing and righting themselves, with the movements of the cœlomic fluid.

It is certain that this is one of the species included by Prof. Verrill in his *Clitellio irrorata*, and a few specimens are included among those so labelled. As no type is indicated, it seems perfectly proper to preserve the name by applying it to this, rather than to permit it to lapse as a synonym of one of the species already named, even though the latter chiefly influenced the original description.

Like *M. glaber* this is a brackish water species, and the two are frequently found associated about the outlets of littoral springs where an accumulation of organic mud makes the conditions otherwise favorable. The remarks under *M. glaber* concerning acclimatization to fresh and salt water apply equally to this species, which is, however, much less resistant to unfavorable circumstances than that species. Perhaps this fact accounts for its much less abundance, though its habit of burrowing more deeply into the soil among the roots of beach grass, in the smaller rootlets of which its peculiar capillary setæ become twisted, make it much more difficult to find. Sexually mature individuals constitute a much smaller per cent. of those found than in *M. glaber*. So far as now known this species occurs only south of Cape Cod.

Tubifex benedeni Udekem.

Tubifex benedii Udekem, 1855.
Clitellio ater Claparède, 1862.
Clitellio irrorata Verrill (in part), 1873.
Hemitubifex benedii Beddard, 1889.
Psammoryctes benedeni Michaelsen, 1900.

In its usual form this interesting species is at once distinguished from any others of our marine oligochætes by the nearly black or deep gray color, resulting from the remarkable flattened papillæ filled with greenish-gray granules with which the cuticle is thickly studded. These papillæ are arranged in irregular transverse rows in all regions except the prostomium, peristomium and clitellum, but they differ greatly in size and consequently in conspicuousness in different regions, being largest on the segments following the clitellum, and thence gradually decreasing in size toward the posterior end, where they are small and widely separated. There is also much individual variation in respect to the number and size of these papillæ, and it seems probable that they may be shed and developed periodically, though my opportunities for observing this species over a period of time have been very limited. Certain it is that small individuals with nearly or quite smooth cuticle are frequently found living with fully papillated mature ones, from which they appear to be otherwise indistinguishable. Fully grown worms with the papillæ scarcely developed also occur, and some of these are among the material collected by Prof. Verrill at Savin Rock, near New Haven, Connecticut, which is one of the type localities for *Clitellio irrorata*. It is quite evident, from a careful study of Prof. Verrill's description, that his account of the setæ is derived largely from specimens of this species in which, as is frequently the case, some of these organs were broken or abraded, and the others exhibited the normally great variability in the length of the distal process. When as slightly developed as in the specimens mentioned, the papillæ might be readily overlooked as unimportant. In my experience the capillary setæ are more often absent than present. That the European worm also is variable in these respects is shown by discrepancies in the several descriptions, and by the numerous names that have been applied to the species and listed by Michaelsen. So far as studied the internal anatomy of American examples agrees fully with the accounts given by Claparède, Beddard, etc. The spermatophores are very large, but less slender than in *Clitellio arenarius*.

This black worm is widely distributed on the seashores of Europe down to a depth of 7 fathoms. On our coasts it has not hitherto been identified. It is common between tides on muddy shores strewn with stones in Casco Bay, Maine. Here it occurs nearly up to high-water mark in association with *Clitellio arenarius*, though the latter is far more abundant. In similar situations in Narragansett Bay also both species occur, and Prof. Verrill has taken them near New Haven. The writer has found *T. benedeni* only sparingly in the neighborhood of Wood's Hole, and in water both fully salt and brackish. My brother, Dr. H. F. Moore, has collected it along with *Clitellio arenarius* at Campobello, New Brunswick.

Tubifex hamatus sp. nov. Pl. XXXII, figs. 12–18.

Length up to 35 or 40 mm.; greatest diameter about .8 mm. at the genital region; number of somites 85–110. In preserved specimens the prostomium is short and bluntly rounded, the peristomium about as long as the prostomium and divided into two rings, the first of which is papillated and evidently retractile. Succeeding somites increase in size, and the next five or six are biannulate, with the smaller annulus anterior. No further annulation is evident. The greatest diameter is at XI, behind which the segments become much narrower, but undergo no diminution in length for half the length of the body. Many of the specimens have somite VIII and often part of IX or even X strongly wrinkled or furrowed transversely. None has the clitellum distinctly developed. The cuticle is thick and everywhere perfectly smooth.

Setæ are absent from the peristomium, the ventral bundle of XI and the anal somite. Elsewhere from one to four occur in each bundle. Anterior to the clitellum four is the normal number both dorsally and ventrally on somites V to VIII, and usually two or three on the remaining somites. Postclitellar somites bear almost invariably two in the ventral and a single large one in the dorsal bundles. In no case have capillary setæ been detected in the dorsal bundles, the setæ being, therefore, exclusively of the hooked and bifid type. It is, of course, possible that further acquaintance with the species, now known from but one locality, may disclose the occasional or periodical presence of capillary setæ in the dorsal bundle. The anterior setæ (fig. 14) exhibit no noteworthy peculiarities, and are but little larger in the dorsal than in the ventral bundles. They are slightly sigmoid, with a small nodulus at the junction of the inner and outer limbs, the distal process of the slightly hooked tip longer and more slender and the proximal one shorter and stouter. Behind the clitellum they undergo little change for several segments, but at about the 20th to 28th somite in different individuals the dorsal and ventral setæ become strongly differentiated. The latter (fig. 13) gradually diminish in size and the two divisions of the tip become equal in length, the proximal one, however, remaining the stouter. Probably as a result merely of their being less worn, the points are usually longer and sharper than on more anterior setæ. At the point indicated the dorsal bundles become abruptly reduced to a single seta of very characteristic form (fig. 15). These are very large and stout, especially in the outer limb, which joins the inner one in an abrupt elbow marked by a prominent enlargement. The tip remains bifid, but is formed almost entirely by the stout, strongly hooked proximal division, which bears the small, slender distal division as an accessory process on its convexity. In intact individuals such large setæ continue nearly or quite to the caudal extremity, but in those which are regenerating the dorsal as well as the ventral bundles contain unmodified setæ, which are consequently here probably provisional.

The ventral setæ of X, which lie just anterior to the orifice of the spermathecæ, are much modified as copulatory setæ (fig. 17). Usually each fasciculus is reduced to one functional and one developing seta. The former is about one-third longer than the ordinary setæ, with a very long slender outer limb curved in the same direction as the inner limb and deeply cleft into two delicate, very slightly diverging prongs, which are, however, united by a thin curved plate to form a groove. The setæ sacs are also enlarged and their outer thirds form eversible sheaths or pockets for the setæ, and their closed ends receive the secretion of two or three small groups of gland cells.

The preservation of the specimens is unsatisfactory, so that histological details are avoided in the following description of the internal anatomy. The digestive tract consists of a short eversible buccal chamber in I and II, a pharynx with a conspicuous spheroidal and glandular dorsal diverticulum in III, a short œsophagus extending through IV and V, and an intestine with its chlorogogue covering from VI onward. The septa $\frac{III}{IV}$, $\frac{IV}{V}$, $\frac{V}{VI}$ and $\frac{VI}{VII}$ are thickened, muscular, and carried back to the alimentary canal in the form of funnels. The last is especially muscular.

While a true integumental plexus appears to be absent, the vascular system is conspicuous from the great development of the anterior lateral vessels, which are very long and arranged in a series of loops and folds beside the alimentary canal as far back as the genital somites (X and XI), those in the latter especially being enlarged, somewhat moniliform and extending into the sperm sac and ovisacs respectively. The lateral vessels of VIII are also of large size, but whether they form contractile hearts is uncertain. A highly developed gridiron plexus of blood-vessels exists in the walls of the intestine, especially in the somites VIII to XII, where it resembles the condition figured by Stolc for *Bothrioneuron.*

None of the specimens is mature, so that the reproductive organs are not fully developed, and further study may require some modification of the following account, especially in what relates to the penis sheath, prostate glands and extent of the sperm sac and ovisacs. The testes have the usual location in X, but remain small in all of the specimens sectioned. A single sperm sac (which may be double anteriorly) reaches from the septum $\frac{X}{XI}$ as far as XIII or XIV, but probably much farther in fully mature worms. It includes long loops of the lateral blood-vessels of X, and is itself enclosed in the ovisac which arises from the succeeding septum.

The male efferent apparatus is shown semi-diagrammatically in fig. 16. The large, few-celled sperm funnel is so distorted by pressure from the intestines, blood-vessels, sperm sac and the septum $\frac{X}{XI}$ that no accurate drawing could be made. After penetrating the septum $\frac{X}{XI}$ the vas deferens forms several close loops between the sperm sac and the intestine, and then, after curving round the former, takes a more open course in the posterior part of the segment before opening into the atrium. The entire atrium is about one-fourth the length of the vas, consists of a small fusiform sperm reservoir receiving the vas at one end and the rather small prostate gland on one side. At the other end it passes into a scarcely distinct ductus ejaculatorius of about equal length, which in turn passes into the summit of the simple erect penis sac. The latter is about as long as the preceding two parts of the atrium combined, is of nearly cylindrical form, has no special nor well-developed muscle sheath and contains the soft filiform penis, which in these specimens lacks any chitinous sheath. The free end of the retracted penis is received into a small bursa which is provided with a circle of small glands and opens at the position of the missing ventral setæ of XI. The ovaries are in XI, but the oviducts have not been detected. As mentioned above, an ovisac arises from $\frac{XI}{XII}$ and, receiving the sperm sac and a pair of vascular loops, extends to about XV or XVI.

The spermathecæ (fig. 18) open just behind the ventral or copulatory setæ of X. They are probably not fully developed in any of the specimens. In those of largest size they have a length about equal to one-half the diameter of the body, are more or less club-shaped with a simple duct forming about one-half the length, and a more or less distinct pouch, which may be elongated or spherical and pass gradually into the duct or be sharply defined. No distinct spermatophores are present, though several of the spermathecæ contained small elongated aggregations of spermatozoa.

Nephridia are present on the left side only of most, if not of all, segments. In sections they are very conspicuous owing to the large size especially of the middle tubule loop, but they are not sufficiently well preserved to permit a detailed description of their structure. The terminal vesicles are likewise large and the external pores, which are situated well mediad and a little cephalad of the ventral setæ, are very conspicuous in entire mounts, in which their asymmetry is very striking.

The brain (fig. 12) is somewhat broader than long, slightly cleft anteriorly and deeply cleft posteriorly.

This species has been found only under stones between tides on the shores of the Acushnet river, above New Bedford, Massachusetts. At this point the water is brackish.

Limnodrilus subsalsus sp. nov. Pl. XXXIII, figs. 19–22.

Length up to 40 mm., but most examples are less than this; greatest diameter (at XII) .6 mm.; number of segments up to 120.

The prostomium is conical with the apex rounded, one and one-half times to twice as long as broad. The first two or three segments are very short, not exceeding the prostomium; succeeding somites rapidly increase in length to X, which, with those following, is five or six times that length. The greatest diameter is at XI and XII, from which point it decreases to the very slender posterior third. Usually the peristomium is simple, but occasionally is faintly biannulate; II, III and IV are very distinctly biannulate, with a small, sharply defined annulus split off anterior to the setæ, which are borne on the prominent middle portion of the larger annulus; V is triannulate with the setæ posterior to the middle of the largest, middle annulus; VI has a second narrow annulus splitting off anterior to the setigerous one and a single one behind; VII is more distinctly quadriannulate, and VIII has four narrow annuli before and two behind the large setigerous one. The next few annuli present an irregular multiannulate condition, there being in most cases 5 presetal and 2 postsetal rings, which are nearly or quite equal to the setigerous one. Postclitellar somites are only very obscurely or not at all annulated.

Setæ are absent from the prostomium, the ventral bundles of XI and 2 or 3 caudal somites. Preclitellar bundles contain 4 to 6, usually 5 setæ; behind the clitellum are found at first 4, then 3, and toward the posterior end 2. Smaller numbers are very likely to occur in the dorsal bundles, though there is no constancy in this respect. There are no copulatory setæ and none in any way much modified or enlarged. Throughout the entire length they have essentially the form shown in figs. 19 and 20, the ventral ones averaging somewhat longer and stouter. The two divisions of the tip are nearly equal in length, especially in posterior setæ, but the distal is usually longer and the proximal stouter.

The simple digestive tract has the pharyngeal diverticulum divided into two lobes by a median fissure in II and III; the chlorogogue investment begins in V or even IV, and the posterior region of the intestine is very strongly beaded. The anterior margin of the brain, near which the connectives arise, is nearly truncate, but has two pairs of small ganglionic projections; the posterior is about one-half as wide and produced into a pair of prominent lobes separated by a narrow sinus, through which the dorsal vessel passes. Nephridia are of the usual type, symmetrical, and open immediately in front of the ventral setæ.

The chief characteristic of the species is found in the reproductive organs, especially in the abruptly bent atrium. The testes are in X, the ovaries in XI, the latter filling a large part of the cavity of that somite, but apparently unprovided with an ovisac. The former produce spermatogonia very copiously and fill not only the cavity of X, but a prominent median sperm sac which reaches to about XXI. The male genital ducts (fig. 21) present the structure and complexity of Eisen's genus *Camptodrilus*. The large discoid sperm funnel is in contact with anterior face of the septum $\frac{X}{XI}$, on the posterior side of which the vas deferens has contracted to a diameter of one-eighth to one-tenth its diameter. From this point the vas deferens forms many and varied coils and loops (not accurately represented in the figure) and has a total length to the point of entrance into the sperm reservoir of about three and one-half times the entire atrium. The sperm reservoir (*ss.*) is stoutly fusiform, constitutes nearly one-fourth of the entire length of the atrium and receives the massive prostate gland (*p.*) near the middle of the ventral side. A ductus ejaculatorius about as long as and one-fourth to one-fifth the diameter of the reservoir unites the latter with the penis sac. Sometimes this ductus is folded as in the figure, and has a posterior connection with the sperm reservoir; in other examples it stretches forward beside the penis sac, and the ends of the reservoir are reversed. The penial apparatus is bent sharply at the summit of the bursa at nearly a right angle and extends thence caudad, sometimes horizontally, sometimes obliquely upward, carrying with it the septum $\frac{XI}{XII}$ to a point opposite the setæ zone of XII. The penis is the direct continuation of the vas deferens, and is slightly bulbous at the distal and gently enlarged toward the proximal end. Surrounding it is the cuticular penis sheath, which is 12 or 13 times as long as its proximal diameter and closely envelopes the penis except at the distal end, where it expands broadly like the mouth of a trumpet. The epithelial penis sheath (*es.*) expands distally into a bursa divided by a horizontal, diaphragm-like partition into a larger dorsal chamber (*dc.*) which receives the free end of the penis and its cuticular sheath and a very shallow ventral chamber (*vc.*) lined by enlarged cells. From this lower chamber a narrow passage leads to the exterior. The entire penial apparatus is ensheathed in muscle, for the most part arranged in two layers, wound spirally in opposite directions and united at the proximal end in a loose coil around the lower end of the vas deferens. Distally these muscles partly unwind and form a sheet enveloping the bursa and binding the entire organ to the body floor.

Spermathecæ (fig. 22) open as usual in line with the ventral setæ of X. They are usually bent into a coil and have a large spherical pouch and a duct about twice as long, the proximal half of which is narrow and the distal half expanded into a somewhat fusiform enlargement with thick walls.

This species occurs in considerable numbers along with *Tubifex hamatus* under stones at half-tide on the Acushnet river, above New Bedford, Massachusetts. At flood-tide the water is here strongly brackish, and this species is of interest as the first of its genus to be recorded as occurring under such conditions, all other species being inhabitants of fresh water strictly.

Enchytræus albidus Henle.

Enchytræus albidus Henle, 1837.
Halodrillus littoralis Verrill, 1873.
Enchytræus humicultor Vejdovsky, 1879.
Enchytræus littoralis (Verrill) Smith, 1895.

This, the best known and most generally distributed of our littoral Oligochæta, was redescribed and identified with *E. humicultor* Vejd. by Smith. Michaelsen, to whose monograph reference is made for the complete synonymy, considers the latter to be part of *E. albidus* Henle. It is an abundant species from Casco Bay, Maine, to Sea Isle City, New Jersey, at least, and, while found more or less everywhere in the upper littoral zone, is especially abundant in the windrow of eel grass which traces the line of high-water along the beach. Wherever the eel grass is kept moist by brackish water and retains a thick coating of diatoms to serve as food, these worms become large and stout, attaining an inch in length and a millimeter in diameter. Elsewhere they are smaller. The same species, but of smaller size, is found in moist spots on farm lands on Martha's Vineyard, where it could readily be introduced in the large quantities of eel grass that are annually spread for fertilizer. About Wood's Hole it also lives in damp, sandy woods and on the shores of fresh-water ponds, especially of one that formerly was connected with the Sound. Whether this particular species originated on the strand and migrated landwards or *vice versa* is not apparent. Its wide distribution along the shore, however, may be accounted for by the ease with which it could be transported in masses of eel grass attached to floating logs, or by clinging to the feet of migrating shore birds. The same influences would affect *Lumbricillus*, but not the various species of tubificids, which burrow in the mud or conceal themselves beneath stones. As a consequence many of the latter appear to occur quite locally.

Enchytræus albidus may be easily recognized among our littoral species by its milk-white color and nearly straight, internally hooked setæ.

Lumbricillus agilis sp. nov. Pl. XXXIII, figs. 23–28.

The length of fully extended mature worms is about 16 mm. or less, the greatest diameter is .4 mm., and, owing to the relatively stout anterior and slender, tapering posterior parts, the general form is distinctly clavate, particularly in fixed examples. Considerable variation in the number of segments has been observed, ranging from 30 to 48, 47 being a very frequent number in breeding examples. The prostomium is short, blunt and verrucose, and possesses a distinct cephalic pore a little behind the apex on the dorsal side. The spermathecal pores are inconspicuous slits facing laterally in the furrow $\frac{\text{IV}}{\text{V}}$, and the spermaducal pores are rather conspicuous simple or usually trifid slits in the position of the absent ventral setæ of XII. In preserved worms they are usually on the apex of the everted male bursa. The female genital pores are visible only in sections. When fully developed the clitellum is thick and conspicuous and extends completely around somites XI and XII. Anteriorly the somites increase in diameter and length to the genital region, but are always short; posteriorly they taper rapidly, and are slender and divided into 3 annuli, of which the middle one bears the setæ, and each of which may be further divided into 2 or 3.

As usual in the genus, the setæ are of a gently sigmoid form with rather acute, slightly hooked tips and a slight thickening at the junction of inner and outer limbs. Those of the ventral fascicles (fig. 23*a*) are decidedly larger than the dorsal ones (fig. 23). Ventral bundles contain from 5 to 8, usually 6, anterior to the clitellum, and from 4 to 6, usually 5, posteriorly; dorsal bundles usually 5 anteriorly and 3 or 4 posteriorly. On somite XII there are no ventral and only 1 to 3 dorsal setæ.

Anteriorly the color is a delicate pink which resides in the ovaries, testes and œsophagus; this is purest as far back as the setæ of somite VI and again in XI and XII, but is elsewhere obscured by the brown color of the chlorogogue cells. In the youngest worms the blood is colorless, in larger ones sulphur yellow, and in still larger ones reddish-yellow. At all ages these worms are transparent and especially so when young, rendering the anatomical study of living ones an easy matter.

The septa $\frac{\text{IV}}{\text{V}}$, $\frac{\text{V}}{\text{VI}}$ and $\frac{\text{VI}}{\text{VII}}$ are thickened and $\frac{\text{III}}{\text{IV}}$ only less so. The three most anterior bear large, clear septal or pharyngeal glands, the third being more granular than the others. The brain (fig. 24) is slightly longer than broad, straight or slightly truncate anteriorly, with the prostomial nerves and circumœsophageal connectives arising from the antero-lateral angles; just behind these is the narrowest part, from which the width gradually increases to the two quadrate posterior lobes, which are separated by a moderate cleft and give rise to a pair of muscle strands. Copulatory supra-neural glands are well developed, especially in somites III, IV and V. They are slender and elongated, not closely united with the ventral nerve, and open on each side nearly at the ventral setæ bundles.

The blood vascular system presents the usual simple structure found in species of this genus. There is a well-developed periintestinal sinus terminating anteriorly at VII. The dorsal vessel is conspicuous and contractile for most of its length, becomes free from the intestine in XIII, and terminates without bifurcating at the anterior margin of the brain, where it joins the pair of vessels arising from the two loops into which the ventral trunk splits in III. Cœlomic corpuscles (fig. 25) are of two kinds, much the most numerous being elongated, irregular, flattened, colorless and finely granular ones measuring about .025 mm. long and .008 mm. wide. The other and less numerous being flattened, irregularly circular disks, with large granules and a pale grayish-green color, which are .015 mm. in diameter.

Nephridia of the form shown in fig. 26 occur regularly in pairs in every somite, except XI, XII and XIII, from VII caudally. They have very small funnels and massive tapering postseptal regions, from the postero-ventral angle of which the large efferent duct, which is shorter than the massive portion, arises and passes to the external pore.

When fully developed the testes, which occupy the usual position in

XI, are divided into from 10 to 20 slender, pyriform lobes which fill a large part of the somite. Sperm sacs are either altogether absent or very small. The sperm funnels occupy a great part of XI, are slender, about 6 to 8 times as long as thick, nearly cylindrical, more or less folded when *in situ*, and slightly contracted near the mouth, the margins of which are provided with a ciliated roll or lip. The vas deferens is about three times as long as the funnel, closely but very variously folded in XII, and has the terminal part somewhat enlarged and ciliated. It opens into the small, depressed, spheroidal, glandular and opaque atrium, which itself opens on the medial side of a small bursa in the position of the ventral setæ. The bursa can be everted as a conical penis (fig. 27).

Somewhat like the testes, the ovaries are subdivided into about 20 ellipsoidal bodies, each with a cross division, on one side of which is one or several large ova, and on the other a number of small ones. A small ovisac pushes back from septum $\frac{\text{XII}}{\text{XIII}}$, but is never extensively developed. The spermathecæ (fig. 28) are small, pinkish, pyriform tubes without diverticula or distinct ducts, which communicate with the lumen of the œsophagus in V and with the exterior in the furrow $\frac{\text{IV}}{\text{V}}$, near which they bear a circle of glands chiefly aggregated into an anterior and a posterior group.

This is an exceedingly pretty and active little worm which crawls rapidly and clings closely to surfaces. It is extremely abundant among the eel grass thrown on shore near high-water mark, and which accumulates in great quantities in sheltered coves. Its special habitat is a certain stratum in the bedded masses where the plant is neither soaked in water and much decayed, nor dried by the sun and air as in the uppermost layer, but where it remains moist and coated with a layer of diatoms on which the worm feeds. If sexually active worms be removed from such conditions and placed in clean salt water without diatoms the genital organs quickly shrink, but if kept in even a small quantity of moist eel grass exposed to moderate light they continue to reproduce. These worms are much parasitized by a monocystid gregarine. The species is known from Casco Bay, Maine, to Vineyard Sound, Massachusetts.

BIBLIOGRAPHY.

BEDDARD, F. E. On Certain Points in the Structure of *Clitellio*. *Proc. Zool. Soc. London*, 1888, 485–495, Pl. XXIII.

BOURNE, A. G. Notes on the Naidiform Oligochæta. *Quar. Jour. Micr. Sci.*, XXXII (1891), pp. 349–356, Pls. XXVI and XXVII.

CLAPARÈDE, E. Études Anatomiques sur les Annélides, etc. *Mém. Soc. Phys. Genève*, XVI (1861), pp. 101–110, Pls. III and IV.

CLAPARÈDE, E. Recherches Anatomiques sur les Oligochètes. *Id.*, XVI (1862), pp. 217–291, Pls. I–IV.

DITLEVSEN, A. Studien an Oligochäten. *Zeit. f. Wiss. Zool.*, LXXVII (1904), pp. 398–480, Pls. XVI–XVIII.

FERRIONNIÈRE, G. Contribution à l'étude de la faune de la Loire-Inferieure. Annélides oligochètes. *Bull. Soc. Sci. nat. Ouest*, IX (1899), pp. 242–4, 246–9, 294, Pl. XIX.

GOODRICH, E. S. On the Structure of *Vermiculus limosus*. *Quar. Jour. Micr. Sci.*, XXXVII (1895), 253–267, Pls. XXVI–XXVIII.

LEIDY, J. Marine Invertebrate Fauna of Rhode Island and New Jersey. *Jour. Acad. Nat. Sci. Phila.*, III (1855), p. 148, Pl. XI, fig. 64.

MICHAELSEN, W. Oligochæta. Berlin, 1900.

MINOR, W. C. Natural and Artificial Section in Some Chætopod Annelids. *American Journal of Science and Arts*, XXXV (1863), p. 36.

SMITH, F. Notes on Species of North American Oligochæta. *Bull. Ill. State Lab. Nat. Hist.*, IV (1895), pp. 289–292.

VAILLANT, L. Histoire des Annelés. Paris, 1890, III, 2, pp. 421, 422.

VERRILL, A. E. Report upon the Invertebrate Animals of Vineyard Sound and Adjacent Waters. *Rep. U. S. Fish Comm.*, 1873, pp. 622–624, 324.

VERRILL, A. E. New England Annelida. *Trans. Conn. Acad.*, IV (1881), Pl. VIII, fig. 3.

EXPLANATION OF PLATES XXXII AND XXXIII.

PLATE XXXII.—*Monopylephorus glaber*, figs. 1 to 6.

Fig. 1.—Male genital duct entire, dissected. × 180. The histology is semi-diagrammatic.

Fig. 2.—Outline of a living nephridium *in situ*, seen from below. × 180. *v*, ventral blood vessel.

Fig. 3.—Outline of brain, from above. × 180.

Fig. 4.—A ventral seta from VIII. × 335.

Fig. 5.—Three cœlomic corpuscles of different sizes. × 280.

Fig. 6.—A pair of spermathecæ. × 55.

Tubifex irroratus, figs. 7 to 11.

Fig. 7.—Side view of entire male genital duct, the penis sac partly concealed behind the sperm reservoir; from a dissection and sections. × 180.

Fig. 8.—Two spermathecæ from different worms; *a*, much and *b*, little distended with sperm. × 55.

Fig. 9.—Ventral setæ, *a*, from somite IV; *b*, from somite XL, and *c*, from the posterior end. × 335.

Fig. 10.—Cœlomic corpuscles, *a*, of the ordinary form; *b*, the homogeneous, colorless form. × 280.

Fig. 11.—Terminal portion of a dorsal capillary seta. × 560.

Tubifex hamatus, figs. 12 to 18.

Fig. 12.—Outline of brain, from above. × 180.

Fig. 13.—A posterior dorsal seta. × 280.

Fig. 14.—A dorsal seta from VIII, and *a*, tip of a ventral seta from the same. × 280.

Fig. 15.—Two of the hooked dorsal setæ from the middle region. × 280.

Fig. 16.—Outline of the male genital duct, with a portion only of the vas deferens represented. × 180. Funnel diagrammatic.

Fig. 17.—A copulatory seta retracted within its sac. × 280.

Fig. 18.—Outline of a nearly empty spermatheca. The line *a–a* shows the form of the same partly distended. × 180.

PLATE XXXIII.—*Limnodrilus subsalsus*, figs. 19 to 22.

Figs. 19 and 20.—Ventral and dorsal setæ, respectively, from somite VI. × 280.

Fig. 21.—Side view of the entire male genital duct, combined from several dissections. × about 100.

Fig. 22.—A spermatheca shown in outline. × about 100.

Lumbricillus agilis, figs. 23 to 28.

Fig. 23.—Three dorsal and *a*, one ventral setæ. ×335.

Fig. 24.—Outline of brain, from above. ×150.

Fig. 25.—The two forms of cœlomic corpuscles. ×335.

Fig. 26.—A nephridium from life. ×225.

Fig. 27.—Dorsal view of one of the male genital ducts. Drawn from a living worm. ×55.

Fig. 28.—A spermatheca showing communication with œsophagus above and external opening below. ×110.

Monopylephorus parvus, figs. 29 to 34.

Fig. 29.—A strongly bifid seta from the ventral bundle of VI; *a*, a slightly forked one from a dorsal bundle; *b*, tips of three from the posterior region. ×280.

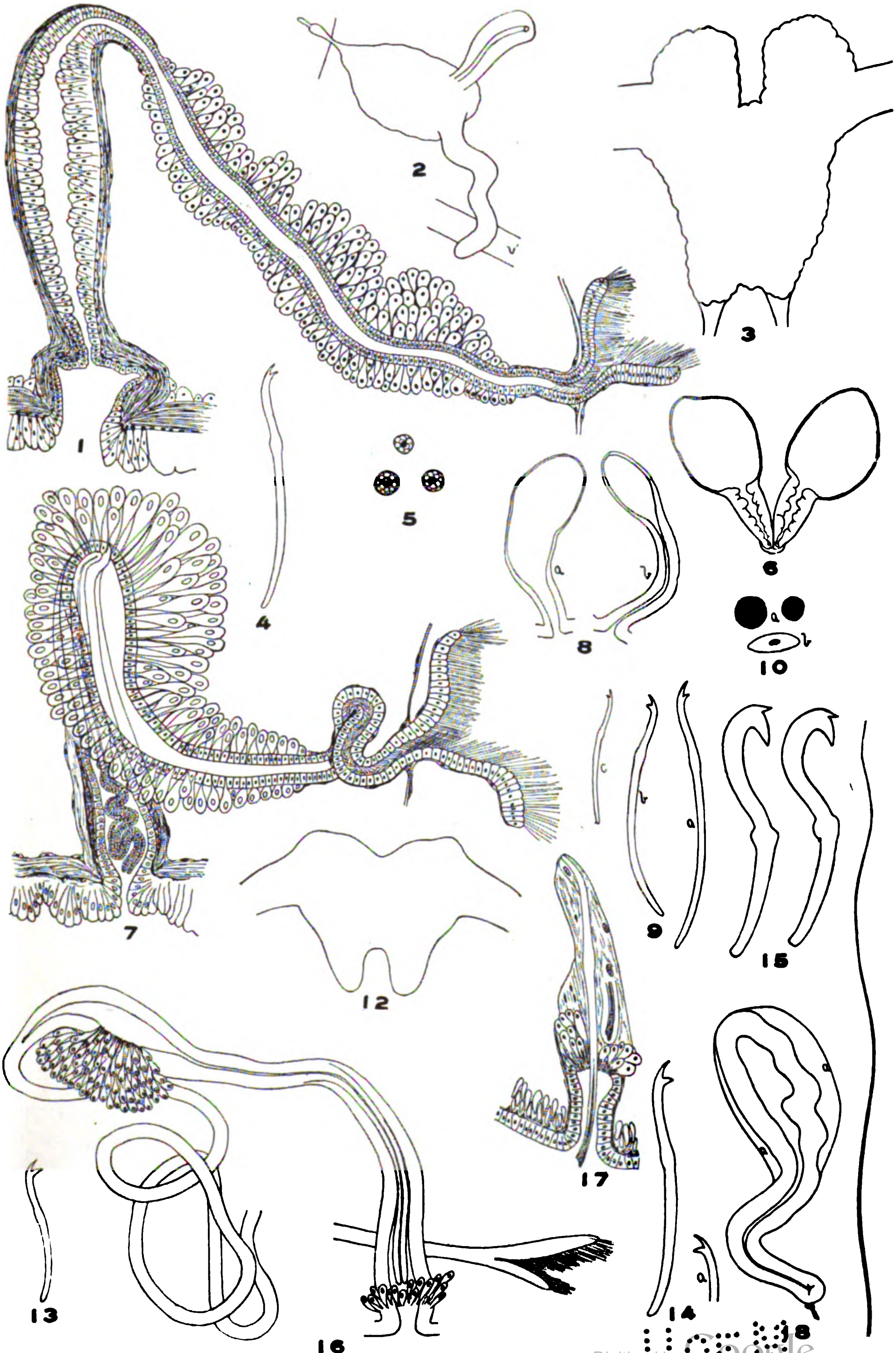
Fig. 30.—Two cœlomic corpuscles. ×280.

Fig. 31.—Outline of the brain, from above. ×180.

Fig. 32.—Outline of both male genital ducts *in situ*, as seen from the ventral surface of the body. ×180.

Fig. 33.—A transverse section passing through the male pore. ×180. *at*, atria; *b*, genital bursa; *dv*, dorsal blood-vessel; *in*, intestine; *n*, nerve cord; *p*, prostate gland; *ss*, sperm sac; ♂, common orifice of male ducts.

Fig 34.—Outline of spermatheca. ×280.



MOORE. OLIGOCHÆTA OF NEW ENGLAND.

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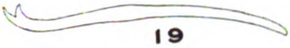
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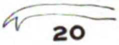
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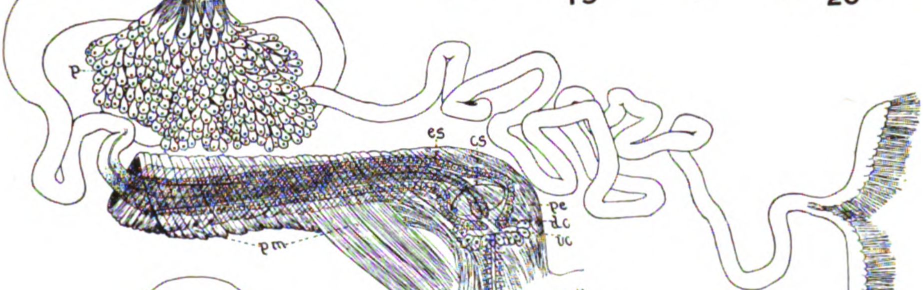

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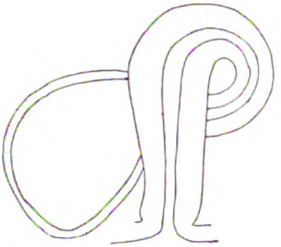
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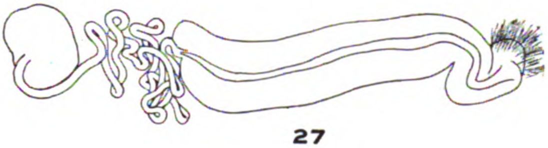
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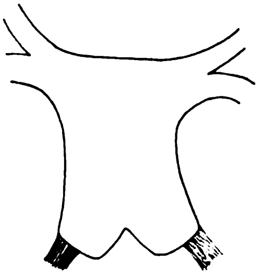
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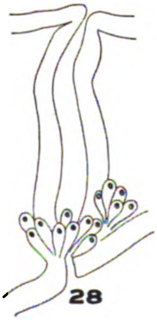
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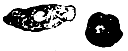
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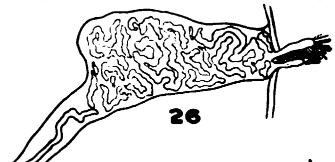
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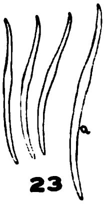

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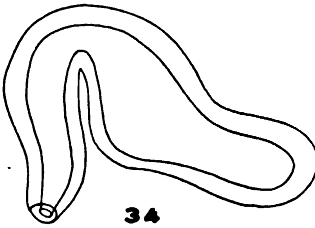
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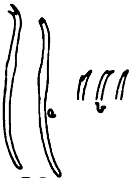

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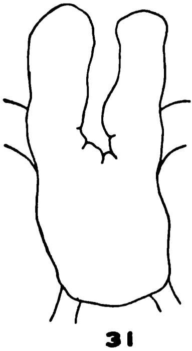
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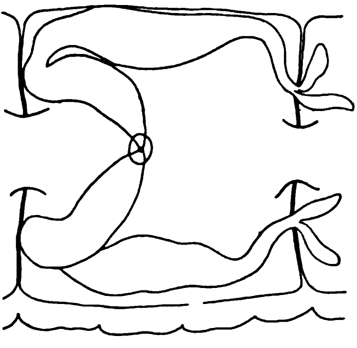
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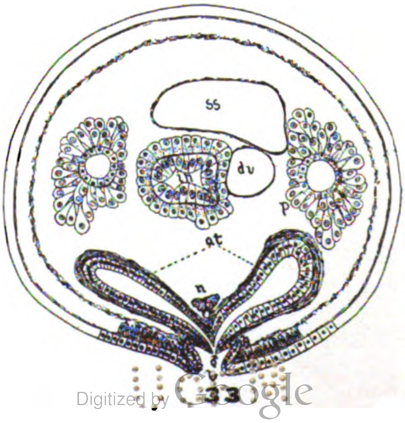

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NEW SPECIES OF POLYCHÆTA FROM THE NORTH PACIFIC, CHIEFLY FROM ALASKAN WATERS.

BY J. PERCY MOORE.

In connection with the investigations of a special Alaskan Salmon Commission the U. S. Fish Commission steamer *Albatross* established, during the summer of 1903, a series of more than 100 dredging and trawling stations, extending from the vicinity of Vancouver Island along the coast of British America and into Alaskan waters as far north and west as Shelikof Strait. The zoölogical collections were in charge of Prof. Harold Heath of Leland Stanford Junior University, to whom and to Dr. B. W. Evermann, I owe the opportunity of studying the annelids.

Some of the more interesting novelties in the families already fully studied are described in this paper; those of the remaining families will be noticed in later papers, while a full report upon the collection, with a complete list of all stations at which each species was taken, will appear in the publications of the U. S. Bureau of Fisheries.

Not the least interesting result of these investigations is the discovery of a probable error of locality in my paper on Greenland Polychæta. In that paper are described four new species of Polynoidæ, viz., *Gattyana senta, Gattyana ciliata, Lagisca multisetosa* and *Eunoe truncata.* Some surprise was expressed at the occurrence of these new species at a single locality in McCormick Bay, while other bottles of specimens from the same bay yielded none of these, but only well-known Arctic species. There was then, however, no reason for questioning the authenticity of the label. The discovery that all of these are common species throughout most of the region covered by these investigations makes their occurrence in Greenland under the circumstances described exceedingly doubtful. Moreover, the collections of this Academy include several bottles of Polychæta taken by Dr. Benjamin Sharp at Icy Cape, Alaska. On seeing the Polynoidæ in question Dr. Sharp states his belief that they were collected by him. It seems almost certain, therefore, that the McCormick Bay label was misplaced in this bottle, and that the specimens really came from Alaska. Unless confirmed by subsequent discoveries the Greenland record should be ignored as valueless.

Eunoe truncata was described from a fragment of the anterior end and proves to have been incorrectly referred. Numerous perfect specimens in this collection show that this is an elongated worm and properly belongs to Kinberg's genus *Hermadion*, if this be recognized as a distinct genus.

Aphrodita negligens sp. nov. Pl. XXXIV, figs. 1, 2; Pl. XXXV, fig. 31.

This species presents a very characteristic shaggy and disordered appearance, owing to the manner in which the loose tufts of coarse dorsal setæ, instead of lying recumbent on or in the dorsal felt, as usual in species of this division of the genus, stand erect and arch high above the back. As a result also of this relation the back appears flat beneath these setæ and the sides are nearly vertical. The general outline is narrowly ovate, the caudal ⅕ attenuated. The extreme lateral margins are formed by the neuropodia and their setæ below, by the convexities of the lateral dorsal bristles above, while the iridescent bristles fall in spreading vertical tufts in the space between but are too sparse to conceal the neuropodial setæ. The dorsal felted area between the bases of the most medial dorsal spines covers about ⅔ of the width of the back, but the spines themselves arch over this area and, as the longest almost meet, form a sort of imperfect bower. As shown below, while there are really but two series of these spines the uppermost tufts are inserted alternately at different angles and consequently perforate the felt at different levels, thus giving an appearance of three series. The segments, of which there are 41 bearing setæ, are strongly defined ventrally, where the body is deeply marked by a median neural groove and a pair of lateral furrows just inside the bases of the parapodia. The anus is small, dorsal, subterminal and posterior to somite XXXVIII.

Numerous minute sharp-pointed cuticular papillæ stud the surface of the body above and spherical papillæ even more thickly below, and the neuropodium as well. Those on the latter vary much in size, being nearly twice as large on the ventral as the dorsal surface of the neuropodium, and especially large ones are clustered about the base of the ventral cirrus.

The prostomium is slightly broader than long, with the pedicle of attachment narrowed dorsally and is smoothly rounded on all sides. There are two pairs of small pigmented eyes, the two on each side being borne on a common slight prominence on the anterior face of the prostomium. On both of the two specimens in hand the median tentacle is missing. The palpi are rather stout for the basal ⅓, then taper rapidly to the slender terminal half, the total length being 4½ times the prostomium. The surface is covered with minute prickles. The frontal caruncle is a compressed, vertical plate as long as the prostomium and much deeper, with its upper border thickened and somewhat lobulated and its ventral margin forming an angle from which an unusually long finger-like process depends over the mouth. The mouth has the usual characters and is bounded posteriorly by III. The dorsal tentacular cirri are about ½ and the ventral about ⅓ the length of the palpi.

Elytra to the number of 15 pairs occur on the usual somites. They are all thin, delicate, smooth and membranous and, except for a slight brown incrustation on the middle ones, quite colorless. Being of large size they cover the back completely and overlap medially. In the middle region they are nearly orbicular and slightly emarginate, but scarcely auriculate as in some species The scar is anterior to the center and reaches to the lateral emargination. Those of the first pair are narrowly ovate, extending over and concealing the head. The next have the part anterior to the pedicle larger than that posterior. Toward the posterior end they become narrower and relatively much longer.

There are 15 pairs of "branchiæ" (fig. 31) also, supported on somites VI, VIII, X, XII, XIV, XV, XVI, XVIII, XX, XXII, XXIV, XXVI, XXVII, XXIX and XXX. They arise from the extreme postero-lateral region of the dorsum of their somites and are rather larger and more complex than in many species. The medial side is high and rises vertically to an angle from which the organ slopes laterally to the level of the back, bearing the often bifid or trifid pinnæ on its free sloping margin. The number of terminal branches varies from 4 on the last and 6 on the first to about 12 on the larger branchiæ of the middle region. The elytra and gills were studied on the type specimen only.

Parapodia are of the usual form. The ventral cirrus scarcely reaches the base of the second row of neuropodial setæ. After tapering for most of its length the cirrus is provided with a subterminal enlargement followed by a constriction and a terminal ball. Dorsal cirri spring from a large ceratophore and the long slender style is shaped distally like the ventral cirri. Like the ventral surface of the body the parapodia are thickly studded with spherical papillæ of various sizes, the larger ones increasing in number laterally and a group of especially large ones occurring on the base of the ventral cirri, while slightly smaller ones are closely crowded about the bases of the setæ.

On cirriferous somites the notopodial setæ are arranged as follows: A dense tuft of not very long capillary bristles springs from the ventral side of the tubercle and spreads ventrally and caudally over and between the neuropodia. The deeper concealed ones are colorless, those on the exposed surface of the tuft iridescent, but they show little brilliancy, being usually of a pale rose-red with dull green or occasionally bluish reflections. Compared with some species they are coarse and rather short, so that they do not conceal the neuropodial setæ. Posteriorly they become dull gray. Immediately anterior to the dorsal cirrus is a tuft of about 7 of the large curved setæ arranged in a transverse row and becoming larger dorsally. A second more median tuft, which springs from a line curving obliquely caudad and mediad, consists of 10 or 11, the ventral ones being very small and the dorsal much larger than any in the more lateral tuft, so that they nearly meet those of the opposite side in the median line. These setæ are of a peculiar clay brown color, of rather soft though brittle texture, finely striated, strongly curved, very coarse at the base and gradually tapered to near the end, which is abruptly contracted to a hard, strongly hooked, acute tip (fig. 2). The surface is devoid of asperities, perhaps through wear. Between these two tufts and anterior to the more lateral a second tuft of iridescent capillary setæ, more slender than those described above, arises and spreads caudally and dorsally of the bases of the large setæ. The dorsal felt fibres arise in a compact bundle dorsal to the dorsalmost tuft of large setæ. The neuropodial setæ are arranged in the customary 3 rows, the dorsal usually containing 2 stout, the middle 3 medium and the ventral 5 more slender ones (fig. 1). All are of similar form, slightly curved and somewhat hooked and tapered at the tip. All of these setæ are much worn, the points blunt and only slight traces of the hairy sheath remaining.

On elytra-bearing somites the arrangement is the same, except that the bundle of felt fibres is wanting and the dorsalmost tuft of large setæ is inclined more medially so that they penetrate the felt nearer to the middle line than on the cirri-bearing somites, leading to the appearance of three series of large setæ, as mentioned above.

At the ends of the body the parapodia are considerably modified. Posteriorly the notopodium becomes relatively more elevated, the whole parapodium more compressed and turned so that it overlaps its successor behind, the capillary setæ tufts more flattened, the neuropodial setæ more slender, the large notopodials more crowded, and the dorsal cirri relatively longer. At the anterior end the first has the usual 3 tufts of capillary bristles, and the dorsal barely longer than the ventral cirrus, the excess being due entirely to the length of the basal joint. The second bears 2 dorsal and 2 middle neuropodial setæ, and a tuft of short fine bristles in place of the ventral tuft of capillary setæ, which is not fully established till about somite VI.

The type of this species is No. 944 in the collection of this Academy, taken by the U. S. F. C. steamer *Albatross* at station 3,727, off Japan. This specimen measures 42 mm. long, 17 mm. wide exclusive of the setæ, and about 9 mm. deep. A larger example, measuring 60 mm. long and 40 mm. wide in a much contracted condition, was taken off Port Townsend at station 4,205. It agrees in all respects with the foregoing description, which is based upon the type, and has the body cavity filled with egg strings.

Aphrodita parva sp. nov. Pl. XXXIV, figs. 3–7.

This interesting species is founded upon two specimens of 6 mm. and 9 mm. long respectively, the latter having a maximum breadth of 6 mm. at XI, which is about the middle of the body. Owing to the deposit which completely covers the body above the general outline is broadly elliptical, but beneath this the form is that typical of the genus though less attenuated posteriorly. Twenty-seven segments bear parapodia

The prostomium (fig. 3) is broadly elliptical with the long diameter transverse and about 1⅓ times the length. The posterior margin is slightly concave; the anterior face bears a slight median elevation for the tentacle, and on each side and slightly above it a somewhat more prominent papilla bearing two minute pigmented eyes. A small compressed frontal tubercle having a smooth surface without lobulations lies between the bases of the palpi. The slender tentacle, which is about 2½ times as long as the prostomium, consists of a rather stout vase-shaped ceratophore bearing a slender tapering style with a terminal knob. The palpi arise close together from the ventral surface of the prostomium; they are about twice as long as the tentacle, relatively slender, and taper regularly from the base to a blunt point; their surface is devoid of cilia. Although the large mouth is situated unusually far forward, being bounded posteriorly by a broad, smooth, unfurrowed lip formed by II, the ventral nerve cord may be seen through the skin to bifurcate to form the circumœsophageal connectives at IV, the usual position of the lip.

Notwithstanding the great breadth when viewed from above, the body when seen from below is really more slender than usual in the genus, the widest part being only about ½ the apparent width and much less than this posteriorly. The cuticle is opalescent and thickly covered ventrally with spherical papillæ. Being thickly covered above with a grayish flocculent deposit the undisturbed animal looks much like a piece of sponge or organic débris. On each side is a broad ridge where the dorsal setæ rise above the felt, each one or tuft supporting a thick vestment of the deposit, which increases its thickness many fold and adheres with great tenacity. When this deposit, together with the superficial layer of felt supporting it, is cleared from the back a deeper clean layer of felt is exposed, through which the elytra are visible.

There are 14 pairs of the latter situated on somites II, IV, V, VII, IX, XI, XIII, XV, XVII, XIX, XXI, XXIII, XXVI and XXIX. Owing to their very small size and the softness of the tissues the position of the last 2 or 3 pairs is somewhat doubtful, but as they seem to occupy the positions usual in other species these determinations are probably correct. This count was made on but one specimen and a fifteenth pair may be expected to occur on better preserved material. The elytra are smooth, thin, transparent and colorless, without markings or papillæ of any kind. The anterior and middle ones are orbicular, with large, narrow scars running from the lateral margin to the center; the posterior five are very thin, folded longitudinally, elongated, overlapping, with anterior attachment and free posterior ends.

The parapodia are well developed and prominent throughout, relatively more so than in most species of sea-mice. Typical parapodia (fig. 4) have a prominent, rather slender, elongated, slightly compressed neuropodium, its end fashioned into three inverted steps sloping upward and outward. Like the ventral surface of the body the neuropodium is studded, especially below, with spherical papillæ. The ventral cirrus arises from a short base somewhat on the posterior surface proximad of the middle of the ventral border. Its style is slender, tapering, balled at the tip, and reaches nearly to the end of the neuropodium. The notopodium is more elevated than usual in the genus and forms a broad tubercle which slopes on to the back and bears posteriorly a prominent ceratophore from which the long slender style of the dorsal cirrus arises. The latter is 2¼ to 2½ times the length of the neuropodium, is slender and regularly tapered for ⅔ of its length, then slightly enlarged and again tapered to a terminal ball with thickened cuticle perforated at the end for sensory hairs. These cirri rise above the felt and their ends often appear above the material deposited on the back. Toward the ends of the body the parapodia become both much smaller and more slender; posteriorly they are directed caudally and their dorsal cirri are much elongated; anteriorly the first is bent forward by the sides of the prostomium and bears a tuft of slender setæ and the tentacular cirri at its end. The ventral cirrus of the second parapodium approaches the dorsal in size and form.

Notopodium and neuropodium are each supported by a stout aciculum, both of which are nearly colorless and the former strongly curved and terminating just below the base of the dorsal cirrus, the latter straight and projecting rather prominently through the apex of the neuropodium.

The setæ are arranged as usual in the genus (fig. 4), but in certain respects resemble those of *Lætmatonice*. The neuropodial setæ are arranged in 3 horizontal rows arising from the three steps on the end of the neuropodium. The dorsal contains 1 or 2 of the stoutest, the middle 2 or 3, and the ventral 3 or 4 of the smallest. While retaining the same general characteristics they differ much in detail. The free portion of the largest (fig. 6) exceeds the neuropodium in length, is nearly straight and uniform in diameter to the distal ⅓, which is very gently curved and tapered to a point encased in a densely hairy sheath terminating in a tuft of very coarse fibres with an often recurved axial prolongation. The smallest ventral setæ are nearly colorless, instead of deep yellow like the larger ones, and much less striated longitudinally. Their shafts are distinctly curved, with a short enlarged end (fig. 5*b*) tapering rather abruptly to somewhat hooked tips with less densely hairy sheaths, often worn away. The middle setæ (fig. 5*a*) are intermediate in character. Usually 1 or 2 setæ of the middle and ventral series are provided with a stout curved spur at the base of the terminal region. The location of such setæ is so irregular and their resemblance to the other setæ otherwise so complete that the possibility of this spur being caducous is suggested, yet this is opposed by the firmness with which it is fixed whenever present.

Coarse notopodial setæ are found in two tufts, one of 8 to 10 anterior to the dorsal cirrus and a more dorsal one of 10 to 12. In each bundle they diminish in both length and thickness from the dorsal to the ventral margin. They are brown, polished, flattened and though standing nearly erect are more or less curved toward the median line. The longest are 3 mm. long and, relatively to the size of the animal, very stout at the base, at first gently but in the outer part rather abruptly tapered to a slender end with minute superficial asperities and a hooked tip (fig. 7). The smaller ones are similar but frequently taper to a point lacking the hook. On the ventro-anterior aspect of the notopodium is a dense spreading tuft of setæ varying in size from the smallest of the dorsal groups to strictly capillary fibres; all taper to capillary ends which lack hooks but are provided with recurved asperities coarser than those on the dorsal setæ. This tuft corresponds to the lateral iridescent hairs of the sea-mouse but is dull and gray in the present species. A very large and dense bundle of felt fibres arises on the dorsum of the notopodium and smaller tufts between and anterior to the large setæ. They form a felt much thinner but otherwise similar to that of the large species.

This species is evidently closely related to *A. intermedia* McIntosh, dredged by the *Challenger* in the West Indies, which is, so far as known, somewhat larger. The latter species is very briefly described from a single specimen, but appears to differ from *A. parva* chiefly in the absence of ocular papillæ and eyes, the form of the neuropodial setæ and their longer spurs which, it is implied, occur on all setæ. Although these two stand apart from the remaining species of the genus they can scarcely constitute a group of greater than subgeneric value. In any case it is interesting to find such a comparatively minute representative of a genus that includes some of the bulkiest of polychætes.

The two known examples of *A. parva* were taken in the Gulf of Georgia at station 4,194, in 111 to 170 fathoms.

Euphrosyne bicirrata sp. nov. Pl. XXXIV, figs. 8–12.

In their contracted state the largest examples are 20 mm. long and 6.5 mm. in greatest width, with 25 setigerous segments, all well differentiated. The body has the usual robust, depressed form with the anterior end broadly rounded and the posterior decidedly narrower and more tapering. The smooth dorsal area is completely concealed by the notopodial setæ, but when the latter are removed proves to constitute about one-fifth of the width of the back. The dorsal caruncle is short, the basal plate reaching to the anterior margin only of V, while the posterior end of the prominent crest extends freely to the posterior end of that segment. A pair of prominent black dorsal eyes is situated at the anterior termination of the caruncle and between them arises the median tentacle, which is two-thirds as long as the caruncle with a very large stout base bearing a suddenly contracted terminal filament forming one-third of the total length. The ventral eyes are smaller than the dorsal, black and closely approximated and just external to them on each side is a minute tentacle having a length of about two-thirds the width of the prostomial area. The palpi are ovoid, the broader end largely free at III and are separated for their entire length by a very deep longitudinal cleft. Posteriorly they overlap the mouth, which is small and bounded behind by the wrinkled triangular lip incising III and IV. There is a well-marked and continuous neural groove and the subanal cirri are fleshy and of the usual form. The cuticle generally exhibits a pale blue iridescence and on each side of the dorsal area is a series of brown spots.

Parapodia are of the usual form and the two divisions are separated by a broad naked achætous space. All three cirri and the branches of the gills (fig. 12) are long, slender and regularly tapering, the latter shorter and more slender than the former. The cirri stand a little anterior to the branchiferous zone, the dorsal a little medial to the first dorsal gill, the ventral just behind the neuropodial setæ and the middle about halfway between. Usually the dorsal cirrus is somewhat longer than the others. On most of the somites the branchiæ number 7 or 6 pairs, 2 of which usually occur between the dorsal and middle cirri and 5 between the latter and the ventral margin of the notopodial setigerous area. As the latter distance is much the shorter the gills occupying it are much more crowded than those dorsal to the middle cirrus. When but 6 gills are present the missing one is almost invariably the second from the dorsal end. No gills occupy the smooth achætous interramal space as in some species. Individual branchiæ are usually biramous and cleft almost to the base; others have 3 branches, in which case one is usually very much shorter than the other two; or they may be simple and cirriform.

The setæ are all glistening white or colorless, giving to these worms a beautiful silvery aspect. They are also extremely long, and as the worms roll up they give the aspect of bristly balls like chestnut burrs or minute porcupines. Those on the dorsal surface are much the longer and conceal the cirri and branchiæ completely from view. In the notopodial palisade the setæ are disposed in about 4 transverse rows. In the second row, counting from the anterior, are found the deeply cleft serrate setæ (fig. 11), but these appear to rarely extend much dorsad of the middle cirrus. The prongs are slender and their inner margins extensively and prominently serrated. Like other setæ they are hollow, with granular contents impregnated with calcium carbonate. The first, third and fourth rows contain only the unequally pronged bifid setæ, those of the third row (fig. 9), while not much stouter, being nearly twice as long as the others (fig. 10), and having much longer prongs. As is the case with the serrate setæ, the longer prong is expanded slightly opposite the tip of the shorter, beyond which it tapers in a slightly curved point. Neuropodial setæ (fig. 8) have the form of the smooth notopodials, and the longest are about the length of the shorter of the latter. At the ventral margin of the bundles they become much shorter with more strongly curved tips. Dried preparations of the smooth setæ show that their cavities are camerated, with septa and telescoped siphuncle-like tubes much like a *Nautilus* shell, but much less regularly.

This species is most closely related to *E. borealis* Oersted and *E. longisetosa* Horst, but is distinguished from both by numerous characters. It was taken from the Gulf of Georgia to Behm canal, in depths ranging from 18 to 134 fathoms.

Euphrosyne hortensis sp. nov. Plate XXXIV, figs. 13 to 16.

Length about 22 mm., width 7 mm., number of segments 35 to 38. The general aspect of the dorsum of this species reminds one of a well-kept garden with a central avenue from which diverge parallel rows of plants with aisles between.

The body has the usual depressed form, flat below, slightly arched above; in contraction the two ends are rounded nearly equally. Below the segments are strongly marked; above they are more obscured by the gills and setæ except in the median space which equals about $\frac{1}{7}$ of the total width and in which each segment is divided into a posterior median and a pair of anterior lateral areas. The caruncle is long and narrow, reaching from II to the posterior margin of VI, with a pair of pronounced lateral longitudinal grooves and a narrow but distinct median ridge or crest, the posterior end of which is free. At the end of the caruncle are the conspicuous, black, dorsal eyes, sometimes two pairs, of which the anterior are the larger, sometimes a single pair of elongated form, or occasionally a single one on one side and two on the other. A median tentacle arises from between the eyes and has a length of barely 1½ times the width of the caruncle; it is 2-jointed, the basal joint stout, the terminal filamentous and somewhat shorter. On the ventral aspect of the prostomial area between the first pair of forwardly directly parapodia is a pair of black ventral eyes, which are frequently coalesced into a single median one flanked by a pair of minute tentacles. The palpi are continuous anteriorly with somites I and II, and extend caudally as a pair of prominent rounded lobes which end freely at IV, covering the small mouth, which is bounded behind by IV and V. A faint neural groove follows the median ventral line to the pygidium, which bears a pair of short, thick, fleshy, grooved, subanal cirri.

The notopodia are completely coalesced with the dorsal surface and their setigerous areas cover most of the back except the median region. The neuropodia project from the sides of the somites, and between them and the notopodia are narrow oblique smooth areas without setæ, into which the lower end of the series of branchiæ bend forward. All three cirri are rather stout and conical. The dorsal is situated at the dorsal end of the branchiferous line, the middle anterior to the latter and about the middle of the notopodial area and the ventral just behind the neuropodial tuft of setæ.

The branchiæ are situated behind the palisade of notopodial setæ, beginning at the dorsal cirrus and extending forward around the ventral border of the setæ into the interramal area. Owing to the relatively short notopodial setæ the gills show distinctly and the body appears more depressed and has a dorsal aspect totally different from *E. bicirrata*. Like *E. heterobranchia* and some other species the form of the gills (fig. 16) varies greatly. A few near the ventral end of the series are often quite simple and cirriform, and from this unbranched form they vary to those with as many as 8 branches, the complexity of the branching increasing with approximate regularity from the ventral to the dorsal end of the series, except that the ventralmost gill is usually much more branched than those immediately above it. The following table shows the number of branches counted on segments of the middle of the body of four specimens, the position of the three cirri being indicated by their initial letters:

D.C. 6, 8, 7, 7, 6, M.C. 6, 6, 6, 6, 4, 2, 3, 3, V.C.
D.C. 7, 7, 6, 6, –, M.C. 5, 5, 4, 3, 1, 1, 5, –, V.C.
D.C. 6, 5, 6, 5, 5, M.C. 5, 5, 4, 4, 1, 1, 6, –, V.C.
D.C. 7, 6, 6, 5, –, M.C. 5, 5, 4, 3, 3, 2, 5, –, V.C.

The 2 or 3 ventralmost gills occupy the space between the neuropodial and notopodial setæ, and it will be noticed that 4 or 5 lie dorsal and 7 or 8 ventral to the middle cirrus. The larger numbers are found upon the larger and the smaller upon the smaller specimens, but it seems probable from a comparison of the number of branches that both divisions and coalescences of the ventral gills occur. Reduced numbers are found also on a few segments at each end of the body. The branches, which seldom arise upon a strictly dichotomous plan, spread widely from a short trunk into a low bush-like form and terminate without expansions in slender, tapering tips.

All setæ are white or colorless except at the extreme tips, which are stained with sulphur yellow. All are translucent, very brittle and hollow, with their cavities filled with the usual granular contents. The notopodials are slightly longer than the gills and of two forms. Deeply cleft, very strongly and extensively serrate setæ (fig. 15) occur in the middle rows of the palisade. The remaining rows have only stout spurred setæ (fig. 14) without serrations, and there are no deeply cleft or bifid setæ without serrations. The spur is always very short, though the internal cavity is deeply bifurcate. Many of these setæ have the middle part of the shaft encircled by many fine parallel canals situated just beneath the cortical layer and more or less completely annular. The neuropodials are somewhat longer and more slender. They have more slender, longer and more curved tips and longer lateral spurs than the notopodial setæ just described and in some cases at least the cavity is not bifurcate (fig. 13). The setæ of the middle region depart remarkably little from the exact forms figured for the three kinds.

This species appears to resemble *E. maculata* Horst rather closely, but is readily distinguished by the position of the middle cirrus, which is between the second and third gills in the latter. From *E. heterobranchia* Jonnson the absence of smooth cleft notopodial setæ especially distinguishes it, while *E. aurantiaca* Johnson and *E. superba* Marenzeller both have a smaller number of gills, longer spurs to the notopodial setæ and other distinguishing features.

This species is much less common than *E. bicirrata*, and was taken at stations 4,272 and 4,274 only, in Chilkoot Inlet, Alaska, in 45 to 73 fathoms.

Eunoe depressa sp. nov. Pl. XXXIV, figs. 17, 18, Pl. XXXV, figs. 19 and 20.

An interesting species of at least partially commensalistic habits. The type is 30 mm. long, 11 mm. between the tips of the parapodia, and 14 between the tips of the setæ at XVII, the broadest somite. The largest specimen is 40 mm. long.

The prostomium is about as long as broad, divided anteriorly by a deep median furrow which reaches half its length; its lobes produced into divergent, widely separated and prominent peaks. Of the two pairs of eyes the anterior are slightly the larger, strictly lateral and at the widest part of the prostomium near the middle; the smaller posterior pair dorsal, less widely separated and close to but distinctly anterior to the posterior margin of the prostomium. All of the cephalic appendages are short and stout. The median tentacle is barely 2½ times the length of the prostomium, its base very short and stout; the proximal ⅔ of the style thick and little tapered, ending in a slight enlargement beyond which it abruptly contracts into a delicate terminal filament; its surface is covered with numerous but short cilia. The lateral tentacles arise entirely ventral to the median, close to the median line and quite inside the line of the peaks; they are stout, taper gently for about the proximal ⅔ and beyond that are contracted abruptly into a terminal filament; the ciliation is sparse. The palpi are stout, short, in all 4 specimens much shorter than the median tentacle, the terminal filament of which they barely reach; their own terminal filament is minute; their cilia are arranged along a few longitudinal lines. The tentacular cirri resemble the median tentacle, which the dorsal almost equals, while the ventral is ¼ shorter. In one specimen the protruded proboscis is short and broad, 7 mm. long and 3.5 wide. The jaws are deep brown, strong and with a prominent lateral ridge on the jaw plate. There are 9 soft papillæ above and 9 below.

Fifteen pairs of elytra are borne on the usual somites by stout elytrophores which are nearly equalled in size by the homologous dorsal tubercles of the cirriferous somites. The elytra (fig. 20) are thick, stiff and of tough, cartilaginoid texture; the margins bear only a few goblet-shaped sensory papillæ and traces of a few or no cilia; the dorsal surface is studded with small, distant, horny spines and much more numerous, smaller, horny prickles; the former are always in the center of round, slightly elevated brown spots and the latter, while themselves brown, are surrounded by a pale ring. The first elytron is circular, the next 11 subquadrate reniform, and the last 2 or 3 broadly ovate, the last with a straight inner margin.

Typical parapodia (fig. 19) are rather long, high and compressed. The neuropodium is prominent and slopes both dorsally and ventrally to the prominent acicular process which is produced into a short soft supraacicular papilla. The notopodium is also large and produced into a prominent acicular process. The ventral cirrus is subulate, with a slender end reaching to about the third row of subacicular setæ. The dorsal cirrus is remarkable for the great length of the ceratophore which is ⅔ as long as the style and in close contact with the dorsal border of the notopodium. The style is short and stout, little tapered and has a subterminal enlargement tapering into a slender tip.

The body is very flat and much depressed, widest near the middle and tapering both ways. Nephridial tubercles exist on all but a few of the most anterior somites; they are of moderate length and are directed dorsally between the bases of the parapodia. The anal cirri are similar to the dorsal cirri and equal to the last 9 segments in length.

Neuropodial setæ are borne in 3 supraacicular and 8 subacicular series, those in the dorsal rows being, as usual, more slender and provided with a greater number of spines. They (fig. 17) are moderately slender with rather elongated ends provided with numerous rows of spines which become larger and more prominent distally; the smooth tip is rather long, slender and but little hooked. The parapodium of I, bearing the tentacular cirri, has a small neuropodium with an acicular process and 2 or 3 setæ. The notopodial setæ (fig. 18) are relatively few in number, much stouter than the neuropodials, the dorsal ones especially being short and stout and the longest ventral ones about ½ the length of the longest neuropodial setæ; they are closely pectinated with ¾ rings of fine spines, leaving a smooth tip somewhat less in length than the greatest diameter of the seta.

The body throughout is colorless, the exposed parts of the elytra mottled with white and rich brown, the caudal cirri, dorsal cirri and cephalic appendages brown with a subterminal white ring and light filament, the palpi only lacking the ring but retaining the white tip.

This species is represented by four examples, the type from station 4,261, Dundas Bay, 8–10 fathoms, and one specimen each from Union Bay, Alaska, and from station 4,270, Afognak Bay, 14–19 fathoms. The latter is labelled "Hermit crab, messmate," and many of the papillæ on the elytra bear 2 or 3 spines.

Antinoe macrolepida sp. nov. Pl. XXXV, figs. 21–23.

This fine species has a length up to 45 mm. with a breadth of 12 mm. and including the setæ of 18 mm.

The short broad prostomium (fig. 21) is about 1½ times as broad as long, smoothly rounded laterally, broadly cleft and bilobate anteriorly and slightly divided for its entire length by a shallow, dorsal, median furrow. The anterior lobes are broadly rounded and largely occupied by the anterior eyes and the cephalic peaks, usually prominent in the genus, are in this species nearly obsolete. In one small specimen of 14 mm., however, they present the ordinary appearance, and, as the eyes are small, it seems quite probable that in the species as here described we have to do with an epigamous phase in which the great development of the anterior eyes has mechanically or otherwise reduced the size of the peaks. The anterior eyes are very large, provided with well-developed lenses, and occupy the entire antero-lateral aspect of the prostomium, facing forward and outward. The posterior eyes appear by comparison minute, and are widely separated on the postero-lateral curvature of the prostomium; in the preserved specimens they are always partly hidden by the bases of the elytrophores of somite II.

The median tentacle is long and slender, 5 or 6 times the length of the prostomium; its ceratophore is more than ½ as long as the latter, and the delicate style tapers regularly to the outer ¼ where a slight thickening occurs followed by a slender terminal filament. It bears a few scattered clavate cilia. The very small lateral tentacles spring from short, nearly spherical ceratophores situated on the ventral surface of the anterior prostomial lobes and partly beneath the median tentacle. Their total length is only 1½ times the prostomium and their slender subulate styles have the terminal half almost filamentous. The palpi are likewise slender and from 6 to 9 times (usually the former) the length of the prostomium. They taper regularly to an acute tip and are marked with longitudinal ciliated lines. The tentacular cirri are similar in form to the median tentacle, but they are much more strongly ciliated and only ⅔ as long. The mouth is bounded behind by a strongly furrowed lip which reaches to III. In a specimen 30 mm. long the proboscis is extended to a length of 9 mm. and a breadth of 3 mm. It is provided with acute jaws of the usual form and bears 9 soft papillæ above and 9 below.

The body is narrow and nearly linear; its ventral width scarcely equals the extreme length of the parapodia, and is practically uniform from somite V to the middle of the body, beyond which it tapers very gently to the pygidium. A strongly marked neural groove runs for its entire length. Nephridial tubercles are first evident on IX and are nowhere much elongated. Dorsally the greatest width is from VIII to XIX. There are 39 somites.

The 15 pairs of elytra are borne on somites II, IV, V, VII, IX, XI, XIII, XV, XVII, XIX, XXI, XXIII, XXVI, XXIX and XXXII. They are remarkably large, overlap broadly both antero-posteriorly and medially, have a soft membranous texture and a weak attachment, so that they are easily detached. The first is subcircular, the next 3 are broadly reniform, and those following, except the last, broadly elliptical; the last is broadly subovoidal with a straight inner margin. To the naked eye the scales appear quite smooth, but a moderate magnification shows the greater part of the surface covered with scattered small, slightly curved, conical spines which become more numerous toward the posterior margin where they are associated with a few slender, clavate cilia. Cilia of various lengths occur also on the free margin of the scales. It appears that in nature the elytra are frequently lost and replaced, and that when detached asymmetrically the remaining one of the pair will increase in size and completely cover the back as far as the bases of the dorsal cirri of the opposite side. Even when both elytra of a pair are symmetrically developed they overlap so broadly in the median line that less than half of their extent is exposed when all are in place.

As indicated above the parapodia are elongated and fully equal the body width. The prominent and slender neuropodia are obliquely truncate distally and have the dorsal angle prolonged anterior to the setæ into a slender acicular process with a free filament equalling the ventral cirrus in length. The notopodium is a low cylindrical process arising rather anterior to the base of the neuropodium and bears the slender acicular process which is not prolonged into a filament beyond the place of emergence of the aciculum. The notopodial cirri have a form similar to the median tentacle, but the subterminal enlargement is rather more prominent and the entire style about ¼ longer so that they reach well beyond the tips of the longest setæ. They bear clavate cilia which decrease in size and number distally and are altogether absent from the distal filament. The ceratophores are small and slightly dorsal and posterior to the base of the notopodium. Ventral cirri arise well distad of the middle of the ventral surface of the parapodium; they have slender subulate styles that barely reach the base of the neuropodial acicular process. Toward the ends of the body the parapodia undergo the customary changes in form, the posterior dorsal cirri especially being very slender, elongated and nearly lacking the subterminal enlargement.

Each ramus of the parapodium is supported by a tapering aciculum, especially stout in the case of the neuropodium, and suddenly reduced in diameter at the end, which is free for only a short distance. All of the setæ are of a pale glistening straw color and to the naked eye or under a hand lens appear to be highly burnished. The notopodials (fig. 23) are relatively few in number and spread radially from a center. The dorsal ones are quite short; the ventrals about ⅔ as long as the longest neuropodials. All are rather stout, nearly straight, with short, bluntly pointed, smooth tips and numerous rows of minute spines extending about half way round the seta and covering about ½ of its exposed portion. All of the neuropodials and especially the supraacicular ones are very long and slender. They are arranged in a dense tuft spreading vertically. The shaft is very slightly curved and the distal thickening (fig. 22) remarkably elongated and at the tip (fig. 22*a*) abruptly contracted into a delicate terminal bristle. All of this region is furnished with numerous short transverse rows of long, very fine hairs which become longer and more spreading at the tip where a number of the longest overlap the base of the terminal awn. The peristomial parapodium bears small tufts of both notopodial and neuropodial setæ.

The general color of the body above is reddish-brown with narrow, light, transverse, intersegmental lines which become broader in some of the paler specimens, in which also a second narrow transverse line may appear across each segment. The dorsal cirri and all cephalic appendages except the palpi, which are pale, are dark brown with a pale subterminal ring. The elytra are pale marbled with brown, especially posteriorly, or with 2 large divided crescentic spots of brown. One particularly beautiful specimen has the body nearly without pigment, and the elytra with large irregular spots and a narrow posterior border of reddish-brown. The ventral surface generally and the parapodia, except their dorsal surface, are unicolor, sometimes clear pale brown, often dark brown or purplish or even nearly black, but always uniform and unspotted and highly characteristic. The neuropodial cirri are always pale and in strong contrast to their dark surroundings.

Many of these large-eyed examples are sexually mature and, as mentioned above, it is probable that they represent the epigamous phase, which the long setæ indicate may be pelagic. The single small-eyed individual is only 14 mm. long and immature, but the neuropodial setæ are elongated and exactly like those of the larger specimens.

A. macrolepida is quite plentiful at the more northerly stations, but occurs as far south as the Gulf of Georgia, at station 4,192, in 89 to 97 fathoms, but also in 293 fathoms in Chatham Strait, from which the type came at station 4,264.

HOLOLEPIDA gen. nov.

The body is much elongated; anteriorly elytra and dorsal cirri alternate in the usual manner, but in the middle and posterior regions all segments bear elytra only; a large free nuchal plate overlaps the prostomium dorsally.

Hololepida magna sp. nov. Pl. XXXV, figs. 24–29.

Unfortunately but a single specimen represents this interesting species. This is in three pieces, apparently making up the entire worm, as they fit together accurately. If this be the case the total length from the anterior margin of the prostomium to the posterior margin of the pygidium is 250 mm., the number of somites 120 and the arrangement of the elytra as indicated below. The form is elongated and depressed, increasing in width to about XXV, then remaining uniform to about the last 20 segments.

The prostomium (fig. 24) is twice as wide as long, omitting the tentacular prolongations, deeply divided by an anterior cleft and a median dorsal furrow, its posterior border straight and concealed and the lateral angles very prominent. Anteriorly it is prolonged into a pair of prominent tentacular processes in the fashion of *Lepidonotus*, though they lie on a somewhat lower plane than in that genus, being slightly ventral to the median tentacle. The two pairs of eyes are very large, occupy the entire postero-lateral portion of the prostomium and their pigment cups are coalesced. Both are blue-black and possess distinct lenses. The anterior pair looks forward, upward and outward and the posterior upward and backward. The median tentacle arises from a large swollen base which fills the anterior cleft, but the style is lost. As noted above, the ceratophores of the lateral tentacles are the anterior prolongations of the prostomium, which nearly equal and lie somewhat ventral to the median tentacle; their styles are slender, about 2½ times as long as the prostomial width, and taper to the tip without a subterminal enlargement. Comparatively very stout palpi crowd the peristomial parapodia; they are constricted at the base, then swollen and again tapered to acute tips; their length is about 1½ times the lateral tentacles; each is marked longitudinally by a prominent broad ridge and lateral impressed lines.

The buccal parapodia are prominent and elongated, reaching directly forward far beyond the prostomium; they are swollen at the base, deeply cleft at the end and bear no setæ. The tentacular cirri have a distinct subterminal enlargement tapering into a filiform tip. They somewhat exceed the lateral tentacles in length. As usual in the family the proboscis is stout and muscular. When fully protruded it measures 21 mm. long, equalling the first 17 somites, and 7 mm. wide by 9 mm. deep. Its surface is strongly wrinkled and its end bears 16 soft papillæ above and 16 below, all nearly fan-shaped and folicaeous in this specimen, but probably collapsed. A pair of stout jaw-plates above and below are subtriangular, each bearing at the anterior angle a blunt claw-like tooth followed on the lateral border by a dentinal ridge. At the base of the proboscis, on the dorsal surface between the bases of the palpi, is a single prominent tubercle.

The peristomium is scarcely evident as a distinct ring, being completely united with the prostomium and succeeding somite. From the dorsum of the latter (II) arises a prominent nuchal fold similar to but larger than that of *Halosydna gelatinosa.* It is subtriangular, attached by its posterior border but otherwise free, and conceals the entire posterior and middle portions of the head. It can be drawn forward so as to cover much more than shown in the figure.

Generally the segments are well differentiated and about 4 times as wide as long. The nephridial tubercles are prominent, begin on somite XII and project outward and backward. Parapodia are much elongated, quite equalling ⅔ of the width of the body. The neuropodia are strongly predominant, broad and truncate at the ends, with a pointed somewhat membranous presetal lobe which conceals the end of the aciculum. The notopodia are small and very slender; they arise from the middle of the dorsal surface of the neuropodia and enclose a slender aciculum. Dorsal cirri arise just above the notopodia, from large stout bases having a prominent glandular lobe. Their styles are long, about equalling the width of the body without the parapodia; they taper regularly, then suddenly to a terminal filament, and reach far beyond the end of the foot itself to the tips of the elongated setæ; no cilia or papillæ are borne on the surface. These cirri are confined to the segments lacking elytra at the anterior end and all are of equal length. Ventral cirri occur on all segments. They arise from about the middle of the ventral surface of the neuropodium and reach nearly to the tip of the presetal lobe. In form they are rather stout and conical with a short terminal filament. That borne on II has a large ceratophore and a style resembling the dorsal cirri in form and size. The next one has no ceratophore and the style is about half as long, while succeeding ones regularly diminish in length until the normal is attained. At the posterior end the parapodia diminish in size and the ventral cirri are relatively much longer. The caudal cirri are similar to the dorsal cirri and equal the last 9 segments.

Elytra are probably more numerous than in any other species of the family. They occur on II, IV, V, VII, IX, XI, XIII, XV, XVII, XIX, XXI, XXIII, XXVI, XXIX, XXX, XXXII, XXXIII, XXXV, XXXVI, XXXVIII, and then on every somite for 79, leaving only 3 or 4 very small somites at the caudal end doubtful, though these certainly bear no dorsal cirri. It is true that a majority of the elytra are detached and lost, but in several cases they remain on 3 or 4 successive segments. There is, moreover, no mistaking the elytrophores, which are thick, prominent and wrinkled, with swollen margins surrounding the scars. The continuous presence of elytra is further indicated by the total absence of dorsal cirri from this region. Even on the cirriferous somites there is a broad dorsal tubercle with a slightly free margin. It is barely possible that this specimen may be abnormal, but the constancy and regular, paired arrangement of the elytrophores render this extremely improbable. A very few cases were noted in which the elytrophores and elytra were of smaller size than usual. The elytra are of a very soft gelatinous texture, of large size, with nearly central scar, and the margin is broadly lobed and so folded as to draw the elytron into a funnel form. Both those of the same pairs and on contiguous somites are curiously adherent over the back, forming a sort of irregular roof. The adhesion results from a gritty substance, perhaps an extraneous deposit, perhaps a secretion, but apparently not due solely to the method of preparation, as other species contained in the same bottle are free from it.

The dorsal surface of the body generally, the head and its appendages and the proboscis are raisin color; the elytra are more or less tinged with the same. Owing to their being distended with eggs the parapodia are dull yellowish. The entire ventral surface is dirty white.

The setæ seen in mass are of a pale yellow color. The notopodials (fig. 29) arise from the base of the prolonged acicular process and spread horizontally. They are very long and slender, capillary, nearly straight, tapered regularly from the base and smooth or with barely discernible marginal denticulations and oblique striations. Although the notopodium is well developed on II, III, and IV, it bears no setæ on these somites; the extreme posterior ones are very small. The neuropodial setæ form a large vertical tuft and are of two forms. Those dorsal to the aciculum have slightly enlarged ends more or less denticulate and pectinate and tapering to simple pointed tips. The dorsalmost are slender with long capillary tips and very fine pectination. The ventral ones are much coarser, with shorter and more conspicuously pectinated tips (fig. 28). The subacicular setæ have bifid tips (figs. 26, 27). They are rather abruptly enlarged at the end, rather stout, strongly curved and conspicuously pectinated. The bifid tip has the stout limb beak-like and the slender one bent toward it or even hooked.

The type and only known specimen comes from station 4,198, in 157 to 230 fathoms, on Halibut Bank, in the Gulf of Georgia.

Lepidonotus robustus sp. nov. Pl. XXXVI, figs. 32–35.

Founded upon a single example, which the label describes as taken from a hermit crab, this species bears a striking superficial resemblance to the similarly commensalistic *Eunoe depressa*. The type is of relatively large size and robust build, measuring 40 mm. long, 13 mm. wide between the tips of the parapodia of X and about 8 mm. deep.

Without including the tentacular prolongations the prostomium is about $\frac{3}{4}$ as long as wide, the posterior border straight and somewhat concealed by a fold of the succeeding somite, the lateral surfaces bulging into ocular prominences behind the middle and converging thence gradually into the lateral borders of the tentacular prolongations. The latter are of the usual form, slightly divergent, and as long as the body of the prostomium. There are two pairs of eyes, prominent, black, circular and lenseless. The anterior are much the larger, situated on the lateral swellings and look outward, forward and upward. The posterior pair are near the posterior border and directed upward.

All cephalic appendages are short and stout and lack cilia and papillæ. The middle tentacle has a short, very stout base somewhat ventral to the tentacular prolongations with which it is largely coalesced; its style is about $1\frac{1}{3}$ times the length of the prostomium with its tentacular processes, and tapers from a broad base to the abruptly contracted tip which is not longer than the basal diameter and scarcely filamentous. The tentacular processes are largely united along the medial face with the base of the median tentacle, their tips only being free for a short distance; the style is similar to the median one, but somewhat more slender, slightly shorter, and with a longer terminal filament having a length of fully twice the basal diameter. The palpi are very stout, bulge widely beyond the sides of the prostomium at the base, and taper abruptly at the end to a short, acute appendage, five longitudinal lines extend for their entire length. On the two sides the dorsal tentacular cirri are of very unequal length, one exceeding the median tentacle, the other being considerably shorter than the lateral tentacle, which is just equalled by the ventral tentacular cirri of both sides; in form they are similar to the median tentacle. The mouth is large with very rugous lips.

There are 26 segments, all of which are broad and stout, and the posterior ones relatively little reduced, though the body is clearly complete. Nephridial papillæ begin on VIII and continue to XXVI; they are elongated, the basal half adnate, the distal half free and curved outward and upward between the parapodia. The caudal cirri are similar to the dorsal cirri, but more tapered and longer, equalling the last 4 segments.

Typical parapodia (fig. 35) are very short and thick, those in the middle region about ⅖ the width of the body. The neuropodium is obliquely truncate with a very short presetal acicular process near the dorsal angle. The notopodium is a small, rather slender lobe on the antero-dorsal face of the neuropodium and is divided into a prolonged acicular process and a setigerous papilla. The ventral cirrus arises from a rather stout ceratophore near the base of the neuropodium, and its style tapers to a slender tip which reaches slightly beyond the end of the ventral border of the neuropodium. The dorsal cirrus has a very large tapering ceratophore borne partly on the postero-dorsal portion of the parapodium and partly on the body wall and reaching beyond the end of the notopodium; the style has the form of the lateral tentacles and extends beyond the tips of the longest setæ. The last dorsal cirrus is borne on the posterior face of its parapodium and tapers more regularly from base to tip than the others. The ventral cirrus of II resembles in form and size the ventral tentacular cirrus.

There are 12 pairs of elytra (fig. 32) situated as usual in the genus. Notwithstanding their large size they fail to cover the broad back, which is largely exposed along the middle. All, including those apparently of the first pair which are detached, are somewhat irregularly quadrately ovate, thick and of a soft, somewhat gelatinoid texture. Their margins are somewhat folded and irregular but without trace of cilia or sensory papillæ. The exposed surface is somewhat irregular, the white spots being somewhat raised, but there are no spines or papillæ of any kind. The narrow, transversely elongated area of attachment is situated nearest to the anterior and lateral borders.

Neuropodial setæ form a dense fascicle arranged in 3 supraacicular and 7 subacicular horizontal series. They vary but little, are rather stout but less thickened at the end than in many species; the pectinated region (fig. 33) is rather extensive and the smooth tip relatively short and strongly hooked. The notopodials (fig. 34) are a close tuft of rather stiff, acutely tapered setæ marked with close transverse rows of fine teeth. The peristomial parapodium bears a notopodial process with a small tuft of setæ on one side, but lacks it on the other.

Except for a median series of dull brown spots, one on the posterior margin of each segment, the body is colorless. The cephalic and caudal appendages are all deep brown with white tips, the dorsal cirri with but little brown and the ventral cirri white. The elytra are reddish-brown and white. No pigment is found along the anterior border or on an extensive area anterior to and laterad of this scar. A transverse area of clear reddish-brown passes from just in front of the scar to the median border and is concealed by the preceding scale; from this a mottled brown and white area spreads to and along the median and posterior borders, the brown predominating medially, where it is marked by small, round, discrete white spots, while laterally the white becomes predominant owing to the increase in size, number and confluence of the spots. On many elytra there is a narrow, scarcely broken posterior border of brown. The round white spots are slightly raised, and in the center of each is a small yellowish opaque pigment spot or excretophore.

The only known specimen was taken from a hermit crab at station 4,291, 48 to 65 fathoms, in Shelikof Strait.

Lepidonotus cœloris Moore. Pl. XXXVI, figs. 36, 37.

This species, originally described from specimens dredged off the coast of Japan, proves to be one of the most abundant and generally distributed species throughout the region covered by these explorations. It represents in the Pacific the widely spread *L. squamatus* of the Atlantic, and in the younger stages resembles that species more closely, but with increasing age and size the fringe of elytral hairs becomes more restricted and the posterior detached tuft is lost. At the same time the differences in the sculpturing and form of the papillæ and the character of the neuropodial cirri become more marked. Many of the specimens are of a nearly black color.

Ninoe simpla sp. nov. Pl. XXXV, fig. 30; XXXVI, figs. 39–44.

This species has much the form, general aspect and usual size of *Ninoe nigripes* Verrill, but none of the examples in this collection reaches the maximum size of the latter. The body is very fragile and no specimen is nearly complete and none represents the caudal end. The most perfect example, hence designated as the type, consists of 160 segments and is 24 mm. long and 1.3 mm. in diameter. Other larger ones, having a diameter up to 2.5 mm., are less complete.

The prostomium has a slightly depressed sugar-loaf shape, with a length about ⅕ greater than the basal width. Posteriorly its median dorsal portion is inserted into an overarched recess of the peristomium and here bears a pair of minute brown eyespots, behind which one or two others are sometimes visible. Within this same recess, but sometimes projecting slightly beyond the peristomial fold, is a minute mammiliform process (median tentacle) with a thickened base embraced by a pair of slight lateral folds. Two symmetrical dorso-lateral longitudinal grooves join the sides of this recess posteriorly, and probably enter the deep slit-like nuchal organs seen on each side by raising or dissecting off the peristomial covering. A similar pair of more distinct ventro-lateral grooves join the ends of a deep transverse slit bounding the palpi anteriorly. The palpi are a pair of small rounded lobes facing each other on each side of a deep longitudinal groove and sunk into a depression on the ventral surface of the prostomium or between the latter and the mouth. A rather small mouth is bounded anteriorly and dorsally by these palpi and posteriorly and ventrally by the peristomium.

Except for the special features already indicated the peristomium is exactly like the following segment and, also like it, is apodous. Anteriorly the body is slightly flattened and increases in width to the end of the branchial region, behind which it again narrows and becomes perfectly terete.

Parapodia (figs. 39 and 40) are absent from the peristomium and following somite. Beginning on III they are at first small and placed at a low level, but gradually rise to a middle height and become more prominent in the branchial region, behind which they again decrease in size, but retain the more elevated position. Throughout they are uniramous, the notopodium being a mere tubercle bearing sensory hairs and entered by delicate aciculæ reduced to mere fibres. The prominent neuropodium divides distally into presetal and postsetal lobes, of which the former is short and broadly rounded and without any appendage to about XX, when a small papilla appears projecting freely from its anterior and distal margin directly outward or slightly ventrad. This increases in size for several somites, but becomes again greatly reduced by XLV. The postsetal lobe is always much more prominent; on the first few somites it is rather narrowly foliaceous and strongly curved dorsad, ending in a blunt apex; by somite IX it has become much broader, fully equals in length the base of the parapodium and bears a small but distinct branchial process on the dorsal border near the end. From this point the branchial process increases rapidly in length and becomes somewhat broad and flattened, while the postsetal lobe itself becomes gradually wider (deeper) and shorter, until by XXII it no longer reaches beyond the presetal lobe. At this point the branchial process fully equals the rest of the parapodium in length and projects prominently directly upward. Both the foliaceous margin of the postsetal lobe and its process have an open structure and are richly vascular and they undoubtedly serve as gills. Posterior to XLV the branchiæ are so reduced that the foot (fig. 40) consists only of a tapering base bearing small conical divergent presetal and postsetal lobes which become still further reduced toward the caudal end.

Anteriorly each parapodium is supported by three black aciculæ which are curved and tapered distally and terminate in an acute pale-colored tip projecting freely beyond the surface. Posterior parapodia have but two (fig. 41). The setæ are of two forms, one winged and with acute tips, the other hooded crochets. The former (fig. 42) have black bases and pale yellow exposed portions, are sigmoidly curved, provided with a delicate wing and taper to very acute tips. They vary somewhat in length, curvature and extent of the border. The crochets (fig. 43) are black or deep brown throughout, strongly striated, very slightly enlarged distally and then contracted to a scarcely hooked tip provided with five teeth, of which the lowermost greatly exceeds the others in size. The hood consists of the usual pair of delicate striated pieces which embrace the end of the crochet and conform to its shape. Most of the setæ are broken, but enough can be made out of a comparison of all the specimens to show that acute setæ alone, to the number of about 12 or 14 arranged in a fan-shaped fascicle, are found in the anterior parapodia. The number gradually diminishes and about the middle of the branchial region crochets appear and continue in association with the acute setæ throughout the middle region, while posteriorly 3 or 4 crochets alone remain.

The dissected jaws are shown in ventral view in fig. 44. With the exception of the mandibles they are dark brown. The maxillæ are slender, very strongly curved and acutely pointed; their expanded and excavated bases are borne on separate carriers which are deeply emarginated about the middle of the outer margin, but have the contiguous but distinct median margin straight. A narrow horny bow extends from the lateral and distal angle of each carrier to the corresponding second dorsal jaw. There are 3 pairs of dorsal jaws. The large posterior pair are broadly arcuate, connected posteriorly with the maxillæ carriers by a narrow bar and bear 5 or 6 acute, strongly curved teeth and a blunt tubercle. The second pair are triangular, with smooth sides and a single stout claw-like apical tooth. The anterior are similar, but with the base rather trapezoidal in shape. Unlike the other jaws the mandibles (fig. 30) are white with longitudinal streaks of pale brown, the distal margin wide and irregularly denticulate, with a prominent lateral angle and the base slender. They are very hard and brittle.

N. simpla differs widely from all described species of *Ninoe* known to me in the form of the gills and the presence of the mammiliform tubercle or rudimentary median tentacle on the prostomium. The branchial process corresponds to the dorsal limb of the gill of *N. nigripes*, *N. palmata*, etc., but the palmate gill of these species is wanting, though its base is represented by the expanded portion of the postsetal lobe.

This species was taken in Behm Canal, Alaska, only, at stations 4,235, 4,236 and 4,238, on muddy bottoms, in 130 to 229 fathoms.

Goniada annulata sp. nov. Pl. XXXVI, figs. 45 to 48.

The length of mature specimens considerably contracted in alcohol is from 70 to 90 mm., the greatest width to the tips of the parapodia (not including setæ) about 3 mm. The prostomium, which is broadly coalesced with the peristomium at the base, is depressed and about twice as long as its basal width or about equal to the first 6 or 7 somites; it is distinctly divided into 5 rings, of which the first equals the total of the others which successively diminish in both length and breadth, the last being minute and apparently sometimes absent. A wavy longitudinal groove on each side of the head is joined by the transverse furrows, which do not correspond above and below, and meets the nuchal organs behind; there is also a faint longitudinal ventral furrow. The apical tentacles diverge diagonally from the tip of the prostomium; the dorsal pair, which are slightly the longer, about equalling the transverse diameter of the last ring. Eyes appear to be absent. The palpi are small, low, rounded, immobile and widely separated on each side of the mouth.

The peristomium is fused with the prostomium above and the second somite below, where also it is produced forward as a rather prominent median lip. In complete sexually mature worms there are from 130 to 160 somites, of which all but 59 or 60 constitute the posterior or genital region. Especially in the anterior region the segments are well defined, smooth and terete, increasing in length to about XXX, and in width more gradually to XL, where they are about 3 times as wide as long; from that point to LIX or LX they remain nearly unchanged in proportions or even somewhat contracted, except when distended with ova or sperm, when they suddenly expand. In immature specimens this change does not take place. At this point there is internally a strong development of radiating muscles to which the constriction is due, and which form a bar to the movement of the genital products forward. On the other hand, the muscles of the body walls are somewhat weakened and one specimen is actually broken in two exactly at this point. This species, therefore, presents a condition similar to that described for *Goniada foliacea,* which it otherwise closely resembles in many respects. The posterior region is somewhat depressed, an appearance that is much exaggerated by the prominence of the parapodia, especially as the body decreases in width posteriorly. In only one specimen is the caudal cirrus preserved; it arises below the anus and is slender, tapering, and as long as the last 9 segments.

Anterior to XXXIII or XXXIV the parapodia are uniramous, behind that point biramous. The former (fig. 45) consist of an elongated neuropodium terminated by a presetal lobe divided by a deep cleft into slender, nearly conical dorsal and ventral lingulæ and an undivided presetal lobe of similarly slender form and nearly equal length. The ventral cirri are prominent, with swollen bases united to the ventral margin of the neuropodium and followed by a slight constriction, beyond which is a short conical style. Dorsal cirri arise a little above and behind the neuropodium. They have prominent but generally relatively slender bases and broadly ovate foliaceous distal portions bent abruptly dorsad. Beyond a gradual increase in size of all parts, especially the dorsal cirrus and postsetal lobe, relatively little change takes place from before backward. The base of the dorsal cirrus is relatively thickest from X to XX. On the first two parapodia the presetal lobe is undivided and the postsetal altogether absent; on the next 3 the latter increases to its normal proportions.

At XXXIII or XXXIV the notopodium appears somewhat suddenly and immediately bears setæ. It consists of an acutely conical presetal lobe and a minute rather ventral postsetal tubercle and is nearly half as long as the neuropodium. From the point of its appearance caudally the notopodium and all other parts of the foot gradually increase in size and become foliaceous, especially the neuropodial lobes and the notopodial cirrus. At LIX or LX the entire parapodium suddenly becomes larger and more folicaeous, without, however, any change in its fundamental characteristics. It is at this point that the posterior genital region with its swimming setæ begins. In a typical parapodium of this region (fig. 46) both notopodium and neuropodium are large and well separated. The former is the smaller and consists of a presetal lobe broad at the base and with a nearly conical terminal portion and a short, broad, presetal lobe situated somewhat ventrally. The cirrus arises from the dorsal base and a little posterior to the notopodium, has a relatively narrow but flattened base bent abruptly dorsad and bearing a broadly orbicular style, the whole slightly exceeding the notopodium in length. The neuropodium is comprised of the same parts as anteriorly, but the lobes are very much broader and more foliaceous in character; the two presetal ones are terminated by abruptly slender tips, while the postsetal lobe is both broader and blunt-pointed, as well as shorter. The neuropodial cirrus is closely united with the base of the neuropodium and tapers to an acute conical point reaching about half way to the end of the neuropodium. The characters of the foot become emphasized to at least LXXX, behind which the parts diminish gradually in size, the dorsal cirri alone remaining prominent to the end but becoming narrower. Numerous large ova or aggregations of spermatozoa are seen in parapodia of this region, but none anteriorly.

Both notopodium and neuropodium are each supported by a single large tapering acicula. Setæ are of two forms—compound in the neuropodium and simple in the notopodium. Both closely resemble those of *G. foliacea.* The former are arranged in a vertical fan-shaped fascicle and vary in number with the size of the neuropodium. They are colorless, with usually strongly curved shafts terminated by oblique sockets, and bear delicate, nearly straight or curved, acutely pointed and superficially granulated blades. They increase in length both ways to the middle of the fascicle and in the posterior region both shafts and blades are greatly elongated, the exposed parts equalling ½ the width of the body, while the longest in the anterior region are not more than ¼ or ⅕ the corresponding dimension. Simple setæ appear with the notopodia and, like the compound, are arranged in a vertically spreading fascicle composed of never more than about 10 setæ, of which 3 or 4 are ventral to the acicula. They are colorless, shorter than the compound setæ and about twice as thick, slightly curved and rather abruptly tapered to an acute point. Like the compound setæ they are superficially granulated and they are much elongated in the genital region.

A majority of the specimens have the proboscis protruded to various degrees, one to a length of 16 mm., but in no case as far as the jaws. It increases slightly in diameter from the base distally, where it may equal 1.5 mm. in diameter. In the best preserved examples it is fluted longitudinally by about 18 parallel muscular ridges. The entire surface is covered thickly with stiff cuticular papillæ arranged rather regularly in longitudinal rows, to correspond with the ridges. These papillæ are of various sizes, varying from .065 mm. to .02 mm. in height, but chiefly divided into a large and a small set, of which the former crown the ridges and the latter occupy the furrows. These papillæ (fig. 48) are colorless and stiffened by a thick striated cuticle, have broad trilobate bases with the paired lobe forward and the elevated portion a broad-based, pointed and slightly hooked cone, the cuticular covering of which has a deep cleft running up the anterior face and terminating in a pore near the apex.

In the retracted state of the proboscis the position of the jaws varies from somite XXXVII to XLII. The jaws are black and very hard, the principal pair nearer together ventrally than dorsally. The latter (fig. 47*a*) bear 3 or 4 large claw-like fangs which diminish in size medially and point caudal. The accessory jaws are of much smaller and variable size, the dorsal arch containing 14 to 16 besides a few very minute ones. They (fig. 47*b*) have bases which are deeply bilobate anteriorly and broadly unilobate behind, and usually bear a pair of equal divergent claws but sometimes 3 or 1. The ventral jaws are similar and 7 or 8 in number and with the others form an unbroken ring. Anterior to this ring is the jaw sheath consisting of a circle of about 18 soft papillæ. The chevron-shaped jaws form lateral longitudinal series of about 20 at the base of the extended proboscis. As the largest ones occur in the middle of each series and smaller ones at the ends, they form elongated elliptical areas tailing out behind where several quite rudimentary denticles occur.

Considerable individual variation is exhibited in the colors, which have resisted the solvent action of the alcohol quite well. Generally the ground is a yellowish-brown, showing a strong tendency to an annular arrangement in the anterior region where the furrows are pale. Posteriorly it is generally darker and more uniform. Most specimens are more or less blotched or even strongly annulated anteriorly with dark purplish-brown, and each segment in the genital region is marked by a, sometimes very conspicuous, pigment spot at the posterior margin in the median ventral line.

Most of the specimens are sexually mature and have the posterior region distended with eggs or sperm. A couple of immature examples 35 mm. long have parapodia of the usual form, but the distinction between genital and pregenital regions is slight and there are no swimming setæ.

Goniada annulata is a quite common species from Halibut Bank, in the Gulf of Georgia, northward to Chatham Strait. There it occurs in 282 to 293 fathoms at station 4,264. The type comes from station 4,235 in Behm Canal, where in 90 fathoms the species occurs at the least depth recorded.

EXPLANATION OF PLATES XXXIV, XXXV, XXXVI.

(Unless otherwise expressly stated all setæ figured are from somite X.)

PLATE XXXIV. *Aphrodita negligens.*

Fig. 1.—Half of the exposed portion of a ventral neuropodial seta. × 56.
Fig. 2.—Tip of an average hooked notopodial seta. × 82.

Aphrodita parva.

Fig. 3.—Head from above. × 24.
Fig. 4.—Posterior view of right parapodium of X. × 24.
Fig. 5.—Ends of three neuropodial setæ; *a*, spurred and bearded seta from middle series; *b*, bearded seta without spur from ventral series; and *c*, spurred seta without beard from the same. All × 250.
Fig. 6.—Bearded neuropodial seta. × 250.
Fig. 7.—End of a hooked notopodial seta. × 360.

Euphrosyne bicirrata.

Fig. 8.—End of a middle neuropodial seta. × 250.
Fig. 9.—Part of a long slender notopodial from a posterior row. × 82.
Fig. 10.—End of a short, smooth and bifid notopodial from the anterior row. × 250.
Fig. 11.—End of a serrate bifid notopodial seta. × 250.
Fig. 12.—Two gills and the middle cirrus (*mc*) from somite XII. × 24.

Euphrosyne hortensis.

Fig. 13.—End of an average neuropodial seta. × 250.
Fig. 14.—End of a smooth-spurred notopodial seta from the posterior row. × 250.
Fig. 15.—Serrate bifid notopodial seta. × 250.
Fig. 16.—Four gills from somite XII. × 24.

Eunoe depressa.

Fig. 17.—A neuropodial seta from the middle of the bundle of somite V. × 98.
Fig. 18.—End of a middle notopodial seta from X. × 98.

PLATE XXXV. *Eunoe depressa.*

Fig. 19.—Posterior view of parapodia from X. × 15.
Fig. 20.—A middle elytron. The larger papillæ are shown as circles, the small horny ones are indicated on a part of the surface by dots and short lines. × 9.

Antinoe macrolepida.

Fig. 21.—The head from above. × 6.
Fig. 22.—An average neuropodial seta, × 56; *a*, tip of the same, × 250.
Fig. 23.—End of an average notopodial seta. × 56.

Hololepida magna.

Fig. 24.—Head from above, showing the much folded first right elytron in place. × 6.

Fig. 25.—Outline of a cirriferous parapodium from an anterior somite without setæ and as seen from behind. × 8.

Fig. 26.—End of a middle subacicular neuropodial seta from XXV. × 56.

Fig. 27.—Tip of the same. × 250.

Fig. 28.—End of a moderately slender supraacicular neuropodial seta from XXV. × 56.

Fig. 29.—An average notopodial seta from the dorsal part of the bundle. × 56.

Fig. 30.—Mandible of *Ninoe simpla* with the stem broken and ridges shown on the left side only. × 56.

Fig. 31.—Gill (dorsal organ) from somite VII of *Aphrodita negligens*. × 24.

Plate XXXVI. *Lepidonotus robustus.*

Fig. 32.—An elytron from the middle region. The stippling indicates the distribution of brown pigment; the white circles are the raised uncolored areas with a central yellow excretophore(?). × 5.

Fig. 33.—End of a middle neuropodial seta. × 98.

Fig. 34.—An average notopodial seta, × 98; *a*, a portion of the same, × 440.

Fig. 35.—Outline of anterior aspect of parapodium X; the notopodial setæ only are represented. × 9.

Lepidonotus cœloris.

Fig. 36.—An elytron from the middle region of a medium-sized specimen. Only a portion of the sculpturing on each region is shown. × 9. *a*, one of the larger papillæ of the middle region; *b*, two of the lateral marginal papillæ; and *c*, three small rough papillæ from near the anterior end of the fringe. All × 56.

Fig. 37.—End of an average dorsal notopodial seta from XI. × 98.

Fig. 38.—A corresponding seta from somite XI of a specimen of *L. squamatus* from Wood's Hole, Mass. × 98.

Ninoe simpla.

Fig. 39.—Outline of parapodium XXV, showing the acicula and the position of the dorsalmost and ventralmost neuropodial setæ. × 82.

Fig. 40.—Outline of parapodium L, showing crochets *in situ*. × 82.

Fig. 41.—An aciculum. × 250.

Fig. 42.—A margined seta. × 250.

Fig. 43.—End of a hooded crochet from a posterior parapodium. × 250.

Fig. 44.—The jaws represented in their natural relations except that the halves of the basal plate are separated. From a dissection. × 56.

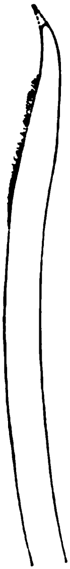
Goniada annulata.

Fig. 45.—Anterior view of a parapodium (XV) of the anterior region. × 32.

Fig. 46.—Similar view of one (C) of the posterior region. × 32.

Fig. 47.—One of the large (*a*) and an accessory (*b*) jaws. × 56.

Fig. 48.—A small and a large papilla from the proboscis, and *a*, one of the latter shown in optical section. × 250.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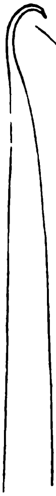
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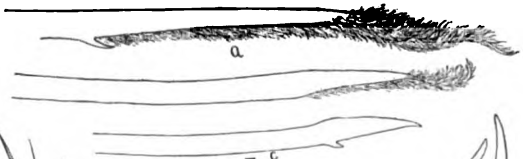

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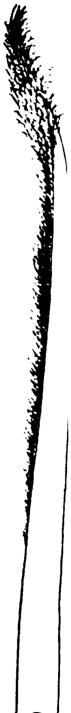
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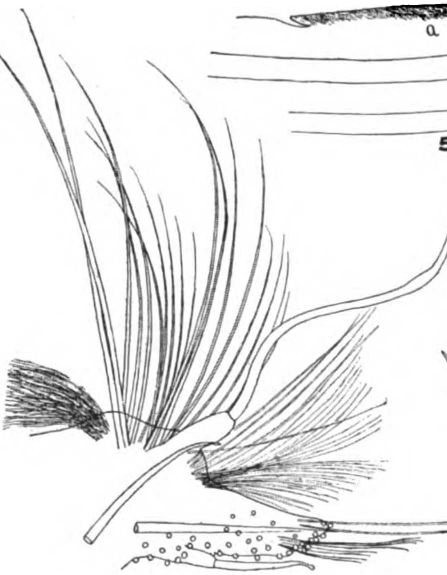
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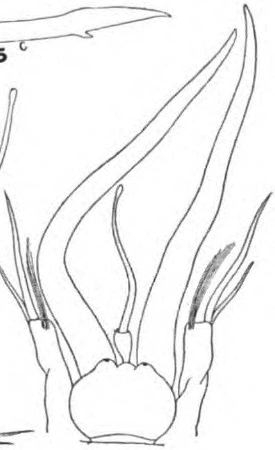
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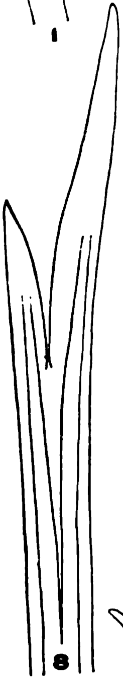
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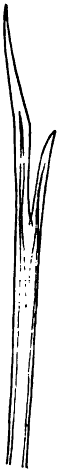
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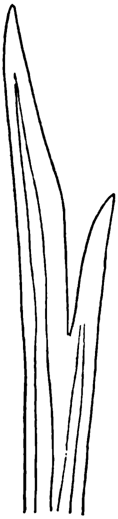
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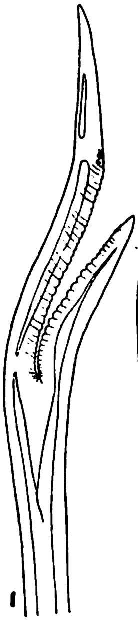
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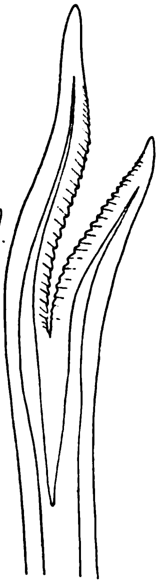
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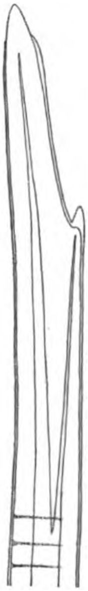
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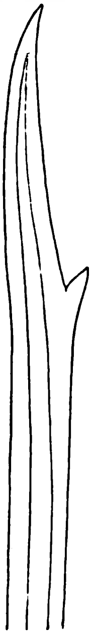
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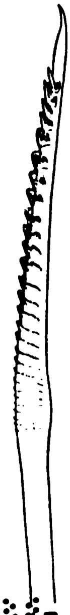
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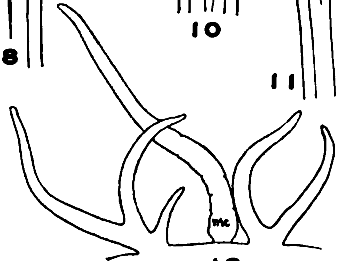

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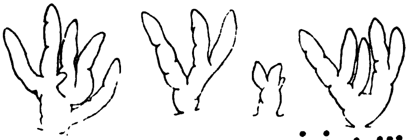
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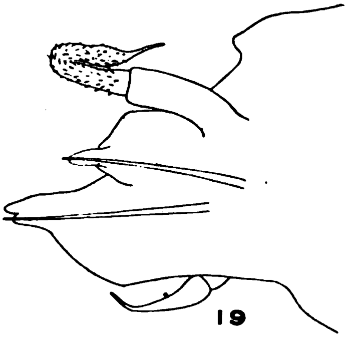

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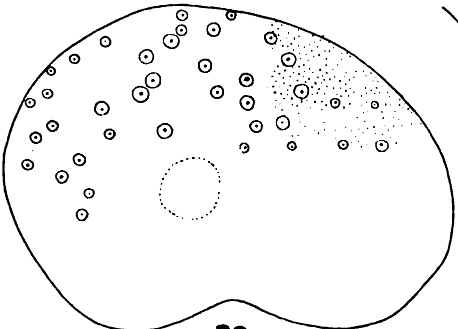

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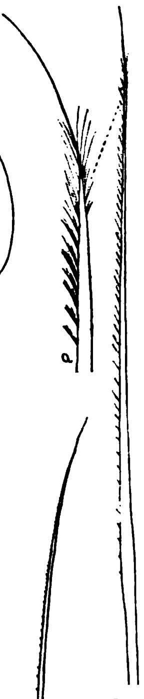

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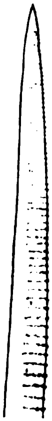

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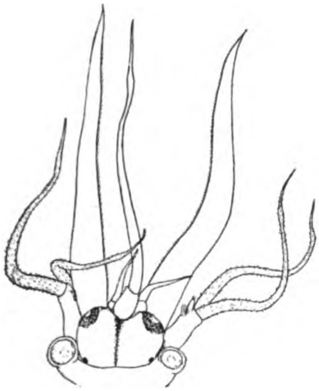

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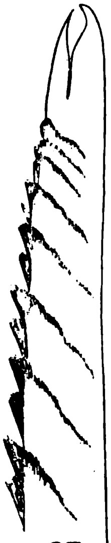

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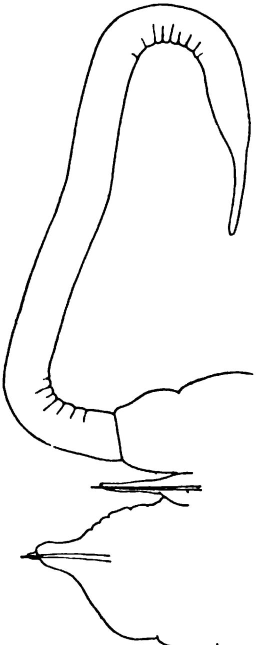

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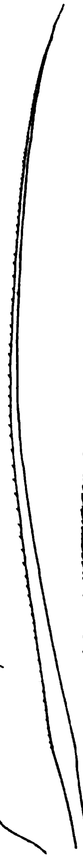

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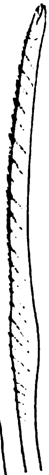

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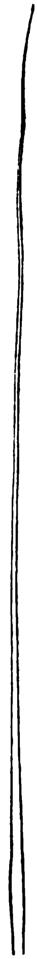

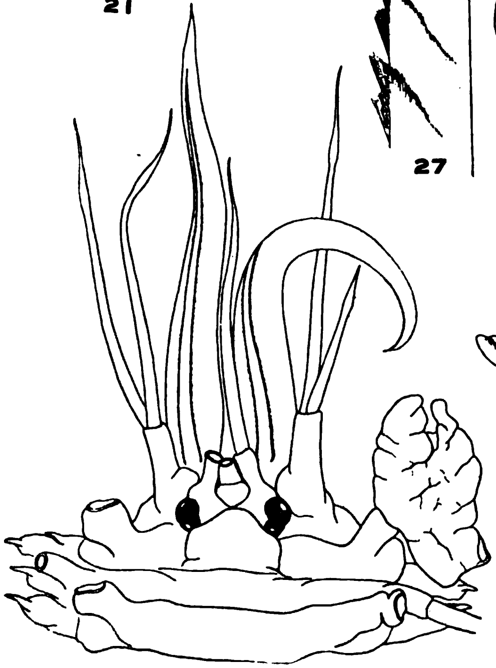

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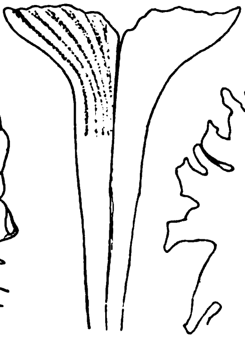

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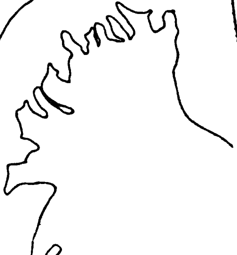

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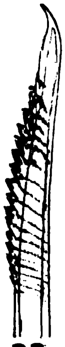

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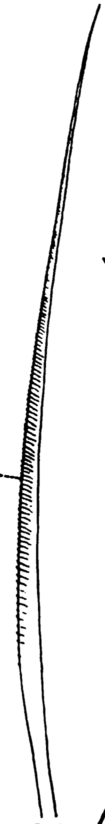

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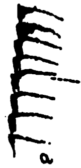

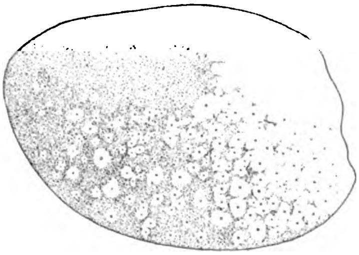

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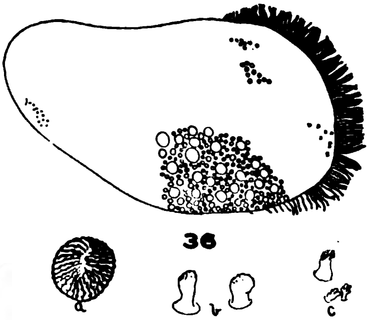

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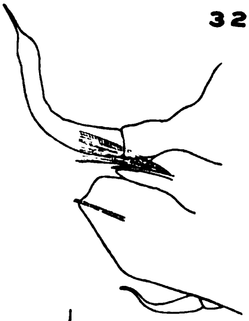

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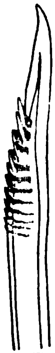

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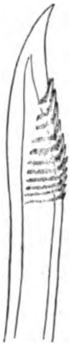

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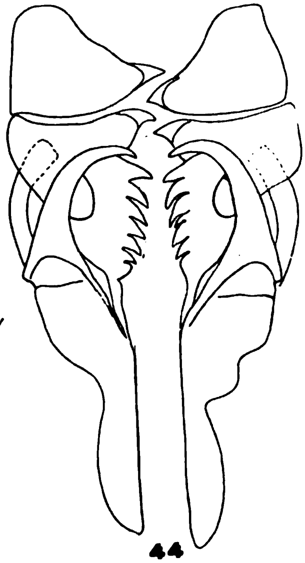

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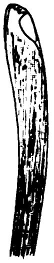

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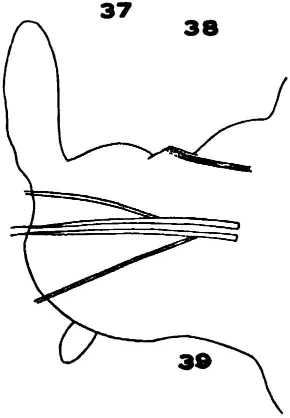

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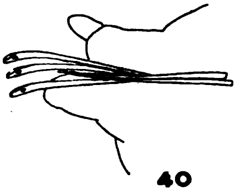

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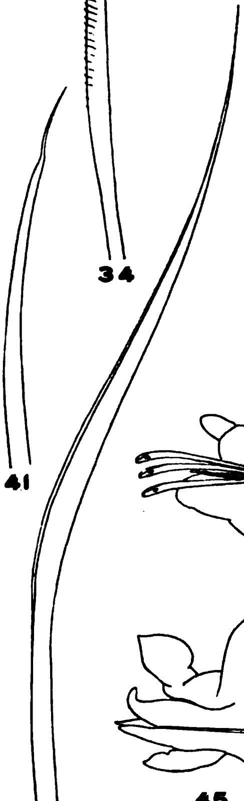

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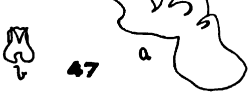

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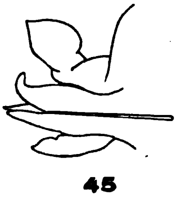

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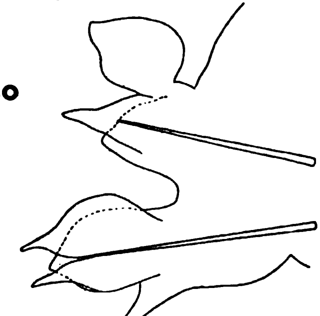

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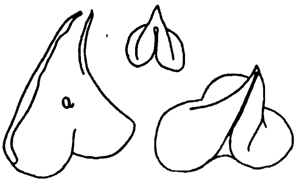

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FIVE NEW SPECIES OF PSEUDOPOTAMILLA FROM THE PACIFIC COAST OF NORTH AMERICA.

BY J. PERCY MOORE.

A noteworthy portion of the Polychæta collections of the Alaskan Salmon Commission of 1903 is seven species, five of which are herein described as new, of the genus *Pseudopotamilla* recently established by Miss Bush. These new species are of interest in indicating the interrelations of *Pseudopotamilla* and other genera into which the former tends to grade. In most respects *P. intermedia* is most typical, and stands between the other species, which may be grouped in couples having quite distinct affinities. On the one hand *P. brevibranchiata* and *P. occelata* approach *Eudistylia* in the structure of the collar, and the former especially in the uncini and the latter in the numerous eyes and general habit. Neither of these, however, shows any indication of spiral coiling of the branchial bases, which are small and simple. On the other hand, *P. splendida* and *P. anoculata* have the angulated branchial stems without eyes and the more elongated spatulate thoracic setæ which characterize *Parasabella* and *Sabella*, but the dorsal collar lobes are exceptionally well developed and the branchial bases are very small and simple. The first three species have the avicular uncini of the posterior thoracic segments enlarged and otherwise peculiar, in this respect resembling *P. oculifera* Leidy.

Pseudopotamilla brevibranchiata sp. nov. Pl. XXXVII, figs. 1 to 7.

A species remarkable for the shortness of its branchiæ and the conspicuousness of its eyes. Two specimens containing nearly mature ova measure 55 and 58 mm. long and have 128 and 151 segments respectively. Of this length the palpi and branchial crown measure only 6 to 6.5 mm. and the thorax 8 mm. Owing to the contraction of the abdomen the form is rather short and stout, but the posterior $\frac{1}{3}$ tapers in the usual way to the small pygidium, which bears a close aggregation of small brown eye spots dorsal to the anus.

The palpi or branchial bases are very firm and rigid, not at all produced ventrally nor spirally twisted, though slightly inflected and winged; the distal margin is even and transverse and the height uniform (about 1.5 mm.) all round. The dorsal free margin bears a rather prominent, rigid, slightly incurved wing which overlaps its fellow medially and is separated by a deep, narrow incision from a similar but thinner wing borne upon the base of the dorsalmost branchial stem. Including a few rudimentary ventral ones and several in process of regeneration at other points, the number of branchiæ varies from 29 to 31. Owing to excessive crowding some of them are forced inward, producing an appearance of two series. The stems or radioles are remarkably short, stout, rigid and brittle and, without considering the rudimentary and regenerating ones, increase gradually in length from ventral to dorsal, the latter being about $\frac{1}{4}$ longer than fully developed ventral ones. Although the outer face is round, the basal $\frac{1}{3}$ or so, distally to the position of the first eye, bears a pair of very low lateral wings or margins which are united for a very short distance at the base. Beyond this point the stems are laterally compressed. The barbs are also thick and brittle, closely two-ranked, and all short, the longest proximal ones not exceeding 3 times the diameter of the stem and the distal ones being much shorter. When fully developed they continue nearly to the end, leaving free a short thick tip only. Eyes are exclusively in one series on the side of the stem nearest to the dorsomedian line. They are deep purplish-brown and very conspicuous, and confined to a zone comprising the middle half of the branchiæ, but most irregularly arranged on individual branchiæ, on which they seldom occupy the entire width of the zone, though they may be variously scattered, or crowded into one or more groups. The usual number has 4 or 5, but varies from 2 to 9, and they may equal $\frac{1}{2}$ the diameter of the stem or be minute, scarcely visible specks, and all or any number may be large or small.

A high oral membrane with rumpled, sinuous margins begins ventrally at the sides of the mouth, is reflected on itself and passes around the internal bases of the branchiæ to become continuous dorsally with the large, much folded, foliaceous tentacles.

The peristomial collar differs considerably from the structure typical of the genus, as exemplified by *P. reniformis* and *P. oculifera*, and indicates the method of transition between forms with the collar lobes widely separated and those with them in contact dorsally. The dorsal portion consists of a pair of very low folds reflected into the dorsal fissure and there coming into contact. These are continuous with the remainder of the collar which rises abruptly into a pair of rounded lobes just above the collar setæ. From this point it is high and regular to near the median ventral region, where a wide open notch on each side partly separates the slender, pointed ventral lobes which are divided from each other by the deep ventral fissure.

Besides the peristomium there are 8 setigerous thoracic somites, all uniannulate and separated by deep segmental furrows. The region is nearly cylindrical, but slightly depressed and from 3 to 3.5 mm. wide. The 8 ventral glandular plates, including the first, are all transversely oblong and the second and third only are divided transversely into 2 equal halves. Abdominal somites are also uniannulate and the posterior ones much crowded. Thick ventral plates are developed throughout the region and are divided into halves by a deep fæcal groove extending from the anus to the first abdominal segment, cutting the latter obliquely to the right and entering the thoracico-abdominal furrow in front of the setæ, then appearing again faintly in the dorsal mid-line of the thorax and entering the dorsal fissure.

Thoracic parapodia are strictly lateral and the uncinigerous tori flush with the surface, while the setigerous tubercles may project slightly or be retracted within little pockets. The collar setæ form a very small tuft and the remaining 7 fascicles are somewhat elongated laterally and oblique. On all thoracic somites the uncinigerous tori are separated ventrally by about ⅙ and dorsally by nearly or quite ½ the body circumference. They increase in length gradually to the third which equals the ventral interspace, then decrease to the last which is ⅔ of the longest, while the first equals the fifth or sixth. As the tori shorten the setæ tufts, closely approximated to their dorsal ends, assume a correspondingly lower position. Abdominal parapodia are more prominent and distinct lateral elevations, highest at the position of the setæ tufts near their ventral ends. The anterior tori are, like the thoracic ones, nearly flush with the surface and are consequently transitional, but, like all of the others, they are separated from the ventral plates by a deep longitudinal groove. Both setæ and uncini are in strictly vertical, linear series. On the first abdominal segment the former has a length of about ½ that of the setæ tuft, and the latter equals the uncinigerous torus of the last thoracic somite. From this point both gradually diminish in length and number of setæ or uncini to the caudal end. At the dorsal end of each thoracic and the ventral end of each abdominal torus is a small, eye-like pigment spot.

In the collar tuft all setæ are capillary, winged, lanceolate, the ventral ones, however, shorter and with wider wings than the dorsal. On the remaining thoracic somites the setæ are of two sorts; a few in the dorsal and anterior part of the fascicle resemble the collar setæ (fig. 1), the number of which decreases from anterior segments backward. Those in the ventral and posterior portions of the bundle form a compact phalanx of several rows of obovate paddle-shaped or spatulate setæ with mucronate tips and the broad blades curved and often slightly asymmetrical (fig. 3). Abdominal setæ are all capillary with the shaft bent at the widest portion of the short double asymmetrical blades. They are arranged in two vertical rows, those in the anterior (fig. 2) having very long, attenuated, smooth tips, the posterior much shorter but otherwise similar. Their number varies from 24 in anterior to 4 or 5 in posterior fascicles. All setæ are distinctly striated.

Thoracic tori bear avicular uncini in the posterior and pick-shaped setæ in the anterior rank, the number of each on somite VI being 65 and on IX 40. The former (fig. 4) have a rather long slender base, narrow but prominent breast, thick erect neck, and little expanded head, with the prominent, finely divided crest pushed well forward and its frontal margin forming a perfectly straight profile with the acute beak, which is just perceptibly bent forward at the tip. On the posterior thoracic somites the uncini are somewhat larger, but the difference is not so great as in *P. occelata* and *P. intermedia;* the neck is also more craned forward and there are other slight peculiarities. The latter (figs. 6 and 7) have slender, finely striated stems slightly curved near the end, and expanded into a coarsely striated head enclosed in a sheath inflated at the base, and prolonged nearly at right angles to the stem into a moderately elongated slender process. Abdominal tori contain avicular uncini only (fig. 5), smaller than the thoracic uncini which they resemble in many respects, but have much the form of the wooden decoys used in duck shooting.

Except for the caudal eyes, the minute spots at the ends of the tori and a trace of brown on the median dorsal portion of the collar, no pigment exists on the body, which is of a pale yellowish color. On one specimen the basal half of the branchial wreath has no pigment, but in the distal half are four partly coalescent bands of brown, not extending on to the back of the stems, but deep-colored on the sides of the latter and the basal half of the barbs. When the branchiæ are opened up the barbs of the concealed branchiæ are found to be of a beautiful orange in those parts from which pigment is absent. The second specimen has very little pigment showing on the exterior except narrow longitudinal streaks of brown on the branchial base corresponding with the intervals between branchiæ. When opened up the distal half of the branchiæ shows traces of orange on the barbs, while the basal half is of a nearly solid orange brown.

The tubes are rather thick, tough and horn brown, and are thickly covered with fine sand; the free end is collapsible for a considerable distance.

This species presents an interesting combination of characters which weaken one's faith in the adequacy of some recent generic definitions. Indeed, it is doubtful whether this species should not go into the genus *Eudistyla*. The entire structure of the collar agrees closely with Miss Bush's description of the organ in that genus; the uncini and setæ are almost counterparts, and Johnson's figures of those of *Eudistyla* (*Bispira*) *polymorpha*, with which the preliminary determination associated these specimens, would answer almost equally well for the present species. The eyes and the dorsal wings of the palpi or branchial bases are also features of similarity. But the absence of any pronounced ventral prolongation of the branchial bases and the total absence of a spiral twist to the latter, in the writer's opinion, more than overbalance those more trivial characters. The shortness of the branchiæ may suggest the possibility of their having been injured and in process of regeneration, but a careful study has brought to light many reasons for rejecting this view.

The type and co-type were taken July 11, 1903, at station 4,247 in Kasaan Bay, Prince of Wales Island, in 95–114 fathoms, in a bottom of mixed mud, sand and broken shells.

Pseudopotamilla occelata sp. nov. Pl. XXXVII, figs. 8 to 14.

From the other species described in this paper the present is distinguished by its great length and numerous and conspicuous eyes. The type measures 130 mm., of which the branchiæ are 18 mm. and the thorax 11 mm. long. A still larger specimen from the same station is nearly 20 mm. longer.

The branchial bases are stiff, high and prominent, of uniform height, provided dorsally with notched wings, and ventrally with a slightly involute thin membrane. Full-grown specimens possess 21 to 24 pairs of branchiæ, small ones 60 mm. long from 17 to 20. They are moderately long, the dorsal somewhat exceeding the ventral, and entirely without a connecting membrane. The stems are rather stout, rounded externally and provided with a slightly raised line on each margin just external to the bases of the barbs. The latter are rather short and well separated toward the base of the stems, but near the distal end become very slender, about three times as long as the basal ones and much crowded, leaving a very short thick tip of the stem which also bears minute budding barbs of decreasing length almost to the extreme end.

Very conspicuous are the rich dark brown eyes, which are very uniform in size and large (about ½ the diameter of the stem), elevated and bulging; all are on the margin of the external surface that lies nearest to the dorso-median line when the branchiæ are spread. They exhibit the usual irregularity in arrangement, but on the ventral branchiæ all are situated on the proximal ⅓ and on the dorsal on the proximal half of the stem, except that on the two dorsalmost they are even more extensively distributed. The ventral stems commonly bear 5 or 6 eyes, occasionally as few as 3, usually distant or in couples. On the dorsal half of the circle of branchiæ each shaft bears from 7 to 12, even the latter number being exceeded on the dorsalmost pair. Except that they are usually much crowded proximally, they are arranged similarly to the ventral ones.

As in *P. brevibranchiata* the collar is intermediate in form between that of *Eudistyla* and typical *Pseudopotamilla*. The dorsal lobes are broadly rounded and slope caudally from their anterior median margin into the dorso-lateral incision; but they are so largely united with the dorsal surface of the thorax upon which they rest that only the outer portion is free, though to a greater depth than in *P. brevibranchiata*. The lateral portions rise abruptly as prominent lobes just dorsal to the collar setæ, and then continue of nearly even height until they rise directly into the elongated narrow and pointed ventral lobes.

Palpal and oral membranes of the usual form are present and the tentacles have a length about equal to the breadth of the thorax, their basal half being broad and foliaceous and the distal half slender and cirriform.

As a result of having been preserved in the tubes the body is slender, elongated and nearly cylindrical throughout, only a very short region at the posterior end being tapered to the pygidium, while the anterior thoracic region is slightly depressed. Probably as a result of pressure in the tubes, the segments are very faintly separated, except along the glandular ventral plates. Except posteriorly they are rather long, the anterior abdominal and the thoracic ones being from ⅓ to ½ as long as wide. The pygidium is oblique, with two pairs of minute lobes guarding the anus laterally and sometimes a group of numerous small brown specks on each side above. Normally there are 9 thoracic segments, of which 8 are setigerous, though one example has but 7. The abdominal segments vary from 125 on a specimen 60 mm. long to 187 on the largest example, measuring 148 mm.

All of the ventral plates are narrow, nowhere exceeding ½ the body width and usually much less than this. Corresponding to the form of the segments the extreme posterior ones are 4 times as wide as long; throughout most of the length of the abdomen they are twice as wide as long; and those of the anterior abdominal and most of the thoracic segments are square, only the first 3 of the latter being again wider and irregular.

Except on the collar the thoracic setæ tufts are oblique and linear, nearly half as long as the corresponding tori, and each guarded by a conspicuous anterior and posterior fold. Tori are relatively short, little exceeding ½ the distance separating them ventrally, and of remarkably uniform length, the second being slightly the longest and those following decreasing in length to the last. On the abdomen the uncinigerous tori and the setæ tufts lie in nearly the same line, the latter very slightly in advance of the former and but little shorter.

The fæcal groove is well marked throughout both thorax and abdomen and passes obliquely across the right side of the first abdominal and last thoracic segments for their entire length.

Collar setæ are all of one form, capillary, acute, curved, narrowly double-winged, not very long. The remaining thoracic fascicles are composed of a small dorsal group of capillary setæ similar to those just described (fig. 8) and a large number of broad-bladed spatulate setæ (fig. 10), forming a close phalanx of several vertical rows. Abdominal setæ are arranged in two vertical rows (about 15 in each on XX), the one with short tips, the other with them more slender and about twice as long (fig. 9). Both have their shafts abruptly bent at the surface of the body, where they are provided with short striated wings of unequal width, beyond which projects the long, slender, acute tip.

Thoracic tori contain the usual two forms of avicular uncini and pennoned setæ, of which there are of each about 45 in each torus of V and 30 on IX. On anterior segments the former have the form shown in fig. 11, except that the beak is usually straighter. The body is long and straight, the breast moderate, the neck rather short, head large and crest prominent and well forward. On the last thoracic segment the uncini (fig. 12) are very much larger and of quite different form, the body being very long and slender, the breast very small, the neck rather long and sloping forward, the head and crest small and the beak less sharply bent downward. Anterior abdominal tori bear about 40 uncini (fig. 13) which are much smaller than the smallest of the thoracic and characterized by the small size of the posterior portion of the body, the large breast, and exceeding high and full crest. The pick-shaped or pennoned setæ (fig. 14) have short stems and slightly enlarged heads with the usual hood and prolonged tip.

Besides a slight tinge of brown about the parapodia, and, on some specimens, 4 to 6 pairs of brown spots, diminishing in size posteriorly, on the dorsum of II to V or VII, there is no pigment on the body. On the branchiæ there is usually a narrow band of dull purplish-brown on the branchial bases and 3 zones of rich purplish-brown on the basal half of the branchiæ, the lower two of which sometimes coalesce to form a very broad zone covering the entire occelated region. On the alcoholic specimens this color very little involves the outer surface of the stems, but is deep on their inner surfaces and the barbs. On one specimen the barbs of the basal half of the branchiæ are also largely orange and the pigment of each eye extends as a narrow oblique line in a proximal direction halfway across the outer surface of the stem.

The tubes of this species occur, sometimes singly attached to stones, sometimes in clumps of several wound among one another in an intricate fashion and firmly united. They are thick, of cartilage-like consistency after preservation and usually little encrusted with sand or other foreign substances. In one case the attached surface of the tubes is much infiltrated with calcareous matter.

Specimens occur off Fort Rupert, Vancouver Island (station 4,202), in 25–36 fathoms, on a bottom of gray sand; in Icy Strait (station 4,261), in 10 fathoms, on a bottom of mud and rock; and at Afognak Island (stations 4,269 and 4,270), in 14–19 fathoms, on a bottom of hard sand with rocks. The last station yielded the largest specimens, among them the type.

Pseudopotamilla intermedia sp. nov. Pl. XXXVII, figs. 15 to 22.

Of this well-marked species the type alone is known. This is a female filled with eggs and having a total length of 58 mm., of which the branchiæ are 3 mm. and the thorax 7 mm. The thorax is 2 mm. and the anterior part of the abdomen 2.5 mm. wide. There are 158 somites, 10 (9 setigerous) of which are thoracic.

Most noteworthy is the small size of the branchiæ, the extreme length of which is only 3 mm. The entire absence of pigment and the relatively pale color of the eyes suggests the possibility of their being in process of regeneration. The branchial bases are remarkably small, of soft texture, have the distal margin evenly transverse, the ventral margin truncate and not at all elongated or spirally coiled, and the dorsal margin provided with a notched lappet as in *P. brevibranchiata*, but smaller and soft and membranous instead of rigid. Each palpus bears 12 branchiæ, including 2 or 3 rudimentary ventral ones. The longest are only 3 times the height of the base, and only a little more than ½ of the length of the thorax. In arrangement they are strictly one-ranked, and none is crowded into the interior. The stems are rather stout, rounded on the external face and lack altogether marginal wings and connecting membranes. The barbs are two-ranked, distributed rather sparsely, the largest about 4 times the diameter of the stem, diminishing in length toward the end, leaving a short thick tip of the stem naked. Never more than 2 eyes, and sometimes one or none, occur on each stem. They are rather small, pale brown, regular in arrangement and always on the proximal half and the dorso-median aspect of the stems of the spread branchiæ. The oral membrane and tentacles are slightly developed, the latter folded longitudinally.

In most respects the collar is typical of the genus. The median dorsal portion is better developed than in *P. brevibranchiata*, but less so than in *P. oculifera* (Leidy); the lateral portions are rather prominent, rising abruptly from just above the collar setæ, and expanding ventrally in broad, prominent lobes separated by the median ventral fissure; on one side a deep notch, on the other a slight one bounds the ventral lobe laterally.

The form is generally slender and cylindrical, the thorax slightly depressed anteriorly, narrower than the anterior region of the abdomen, the posterior half of which tapers very gently to the pygidium, and the anus nearly terminal but surmounted by a small lobe bearing two groups of minute brown eye-spots.

Thoracic setigerous tufts are prominent, the anterior especially elongated and oblique. The uncinigerous tori are very little elevated above the surface, the first and second longest, equalling about ⅙ the circumference of the body and separated by an equal ventral distance. From the second they decrease in length, the last scarcely more than ⅓ of the first and separated by a ventral distance of about ⅙ of the circumference. Abdominal parapodia are rather prominent, especially the ventral setigerous ends, the first about equal to the eighth thoracic and succeeding ones decreasing slowly but steadily to the last.

The ventral glandular plates are narrow on the abdominal region, separated from the parapodia by wide grooves and completely divided into a pair of squares by the deep fæcal groove, which turns to the right obliquely across the first and appears again on the dorsum of the anterior part of the thorax, where it opens into the dorsal fissure. It is not visible on the posterior part of the thorax. The thoracic ventral plates are indistinct and poorly developed in the type.

No pigment is present on the greater part of the body, being confined to 4 pairs of discrete reddish-brown spots on the dorsum of somites II to V, which become successively smaller and the last mere specks. Owing to the presence of great numbers of eggs the abdomen is somewhat yellowish.

All setæ have a pale yellow color. Those in the collar tuft are, like the succeeding thoracic tufts, of two kinds. A few of the dorsalmost are capillary, more or less sigmoidly curved and narrowly winged. The ventral ones, more numerous and arranged in several parallel rows, are of the mucronate-spatulate type, but have a somewhat narrower and longer obovate blade than those on the following somites. On remaining thoracic somites the setæ are more numerous, the capillary (fig. 15) forming a projecting tuft, the spatulate (fig. 17) in more and longer rows and having somewhat shorter and broader blades than those on the collar; the mucronate tip is long and slender. Abdominal setæ are in two vertical rows, 9 or 10 in each row of the first few fascicles and 4 or 5 in the less distinctly 2-ranked fascicles of the caudal end. Both kinds have the shaft abruptly bent just beyond the surface of the body, the longer form with a very narrow accessory wing on the concave side and a wider but very short wing on the convex side, and the smooth acute tip often much longer than in the seta figured. The short setæ (fig. 16) are sigmoidly curved, have shorter, smooth tips and broader, strongly striated wings reflexed from the convex side.

Thoracic tori bear avicular uncini in the posterior and pick-shaped setæ in the anterior row. The former (fig. 18) have long straight bodies, moderate-sized breasts, rather short, erect necks, enlarged heads, prominent crests very low in front, and curved beaks. On the last, and possibly other posterior, thoracic somites the uncini (fig. 19) are very much larger, with very long bodies, minute breast, long neck well sloped forward, head not enlarged, very small crest and weak beak. Another less typical one with somewhat larger crest is shown in fig. 20; 43 occur in the 9th and 60 in the 4th torus, though the latter occupy twice as long a space. The pick-shaped setæ have the form shown in fig. 22, the heads being much reduced and the hoods much inflated and with slender tips about as long as the beaks of the uncini. Abdominal aviculæ (fig. 21) are much smaller than the thoracic, with relatively shorter bases, larger breast, short thick neck, and crest larger, farther forward and extending low down on the beak, which has a straight profile.

One specimen only known, from station 4,267, off Cape Edgecumbe, Sitka Sound, 922 fathoms, on a bottom of soft gray mud.

Pseudopotamilla splendida sp. nov. Pl. XXXVII, figs. 23 to 27.

This large handsome species is founded on two specimens lacking the posterior end; with the 37 anterior segments alone the type measures 65 mm. long, 27 mm. belonging to the branchiæ and 8 mm. to the thorax including the collar, while the greatest width of the thorax, exclusive of the setæ, is 5 mm.

The branchial lobes are very small, barely reaching the border of the collar, stiff, thick, very little free, lacking all trace of the dorsal appendage or lappet of some species; the ventral end lower, not at all produced nor involute, without a thin membrane, and partly united to its fellow of the opposite side. Twenty-two pairs of branchiæ are present in each specimen. Except that those of one side are in process of regeneration all are of approximately equal length and are entirely separate to the base. The external surface of the stems is wider than the internal, flattened and provided with lateral angles but no wings. There is no indication of eyes. The barbs are numerous and closely arranged in 2 ranks; they increase in size regularly from the base for ¾ the length of the stem, those at the distal end of this region being 3 times the length of the basal ones and ½ the width of the thorax; in the distal ¼ they again decrease, leaving a filamentous tip equal to the basal barbs. Several of the basal barbs of the dorsalmost branchiæ are much enlarged.

The conspicuous, flaring collar has very large dorsal lobes, separated by the dorsal fissure and bounded laterally by large, deep incisions midway between the dorsal fissure and the collar setæ; these dorsolateral incisions are partly filled by a small, thin lobe arising from the bottom. The lateral portion of the collar rises in an even curve to the same height as the dorsal lobe, its margin is slightly wavy but not at all notched nor produced into ventral lobes, but instead is broadly rounded below and overlaps the ventral fissure from both sides.

Oral membranes are prominent, but present no characteristic features. The tentacles slightly exceed the collar in length and have a thick midrib tapering to a free end, the basal half being provided on each side with broad margins folded together.

On a specimen preserved outside of the tube the thorax is broad and depressed and tapers regularly from the collar segment into the more slender terete abdomen. The second example, having been removed from a tube after preservation, is more nearly cylindrical throughout. All segments are very distinctly indicated and uniannulate. The type possesses 9 thoracic (8 setigerous) and 28 remaining abdominal segments; another specimen has 10 thoracic (9 setigerous) and 58 abdominal segments, both, of course, being incomplete.

Although the dorsal fissure is deep on the anterior 2 thoracic segments, the fæcal groove is not discernible on the thorax. On the abdomen it is deep and conspicuous, and can be traced around the right side of the first abdominal segment until it disappears in the furrow anterior to the setæ.

The thoracic setigerous tubercles have short bases but project prominently. The tori are very long, the first about twice and the last about 1¼ times the ventral interspace, the others intermediate. On the abdomen the same parts are little elevated and placed at nearly the same level as the dorsal end of the last thoracic. The setigerous line is shorter than the thoracic and the first torus about ½ as long as the last thoracic. About 90 aviculæ and the same number of pick-shaped setæ occur on the torus of III and 60 of each on IX. On the thoracic segments the spatulate setæ are very numerous and arranged in 5 or 6 vertical rows, while the capillary setæ do not exceed about 15. The capillary abdominal setæ are in two regular rows of about 10 each on the anterior segments.

All of the collar setæ and the dorsalmost setæ on the other thoracic segments are rather short, stout, capillary, tapered to an acute tip, little curved and with small, nearly symmetrical wings with very fine oblique striæ on each side. The spatulate setæ (fig. 24) have stout stems and rather long, narrow curved blades about 2½ times as long as wide with apical arms of moderate length. The abdominal setæ of both rows do not differ greatly in length. They are longer than the thoracic capillary setæ, especially posteriorly, and are gracefully curved and tapered, with fairly broad, oblique, strongly striated and nearly symmetrical wings (fig. 23). Avicular uncini are nearly uniform in size and form on all thoracic segments. They (fig. 25) have moderately long curved bodies, full breasts, slender upright necks, elevated crests and slender acute beaks. Abdominal avicular uncini (fig. 26) are smaller and have very small bodies and deeper breasts but are otherwise similar. The pick-shaped thoracic setæ have the form shown in fig. 27.

Pigment is totally absent from the body and on the branchiæ exists only as six regular, narrow zones of pale reddish-brown, chiefly confined to the basal halves of the barbs and scarcely apparent on the stems.

The tubes are nearly straight, thick, pale brown and very little incrusted with sand or other foreign matter.

Known only from two large specimens taken at station 4,245, Kasaan Bay, Prince of Wales Island, June 11, 1903, in 95–98 fathoms, on a bottom of dark green mud and sand mixed with shell and rock fragments.

Pseudopotamilla anoculata sp. nov. Pl. XXXVII, figs. 28 to 33.

This handsome species has the branchial bases exactly as in *P. splendida*, that is, they are very small and concealed by the collar, somewhat coalesced ventrally, and lack the dorsal lappet, ventral membrane and any indication whatever of a ventral involution. There are 15 pairs of plume-like branchiæ, each very long and graceful and without any trace of an interbranchial membrane. The stems are more convex externally than those of *P. splendida,* but still distinctly flattened, and there is no trace of eyes. The barbs are all very slender, 2-ranked, well separated and increase regularly in size from very short ones at the base to very long ones, much exceeding the diameter of the thorax toward the end; in the distal 1/7 they again decrease, leaving a short tip entirely free from them.

The dorsal lobes of the collar are narrow and very long, considerably exceeding the second segment in length, with nearly parallel sides and rounded ends, the two separated by a wide dorsal fissure. The dorsolateral notches are very deep but narrow and not occupied by a small lobe. Laterad of the notches the collar rises nearly to the height of the dorsal lobes, which it somewhat overlaps by means of low, broad extensions toward the median line. The remainder of the margin is even and scarcely crenulate, the ventral lobes arising regularly and gradually, without the formation of any notch, into prominent triangular lobes overlapping the bases of the branchiæ and separated by a deep median fissure.

The single example is fortunately complete. It measures 122 mm. long, the thorax being 15.5 mm. and the branchiæ 32 mm. long, and the former 3.5 mm. wide. It is consequently long and slender, tapering to the pygidium in the posterior 1/3. There are 14 (13 setigerous) thoracic somites and 162 abdominal somites. The former region is scarcely depressed and the remainder of the body nearly cylindrical from pressure of the tube. Besides being unusually broad and deep anteriorly the dorsal fissure is prolonged into a fæcal groove extending for nearly the length of the thorax, but gradually fading out behind. The thoracic segments are 1/2 as long as wide, the middle abdominal about 1/6 as long as wide, and the posterior very much shorter. Ventral plates are developed much as in *P. splendida,* but the thoracic are relatively longer and the lateral notches divide them unequally, being nearer to the posterior border; the anterior abdominal plates are twice as wide as long and the posterior 4 times or more, all very thick and deeply divided; none of the abdominals are notched laterally, and they occupy about 1/3 of the body width.

Thoracic setæ tufts are short and straight, the anterior ones placed at a very high level. The tori of III have a length of nearly 1/4 the circumference of the body and are separated by ventrally 1/2 this distance. From the first they decrease in size regularly, the last and shortest being but ⅓ as long as the first. The first abdominal torus is not more than ½ as long as the last thoracic, and is on the level of the thoracic setæ tufts. The first thoracic torus bears 65 uncini and an equal number of pick-shaped setæ, the fifth 43 and the last 33. Anterior abdominal tori have about 25 uncini and the setæ tufts about 15 setæ, less distinctly in 2 rows than in other species.

All of the collar setæ and the dorsalmost setæ of other thoracic fascicles are of the usual slightly curved, acute, tapering form with short, rather narrow, obliquely striated wings which arise at an angle with the stem. Not over about a dozen occur in each fascicle. Spatulate setæ (fig. 30) are much less numerous than in *P. splendida*, and arranged in only 2 or 3 vertical rows. The wings are decidedly long, the entire expanded region being 3 times as long as wide, with an apical arm exceeding in length its greatest diameter. The striation of the blade is unusually faint. Abdominal setæ (figs. 28, 29) are few (about 15 in anterior tori), not distinctly arranged in two rows, and the longer and shorter ones not greatly different. The stem is constricted but not sharply bent at the base of the blade.

Avicular uncini (fig. 31) of similar form occur throughout the thoracic region. The base is only moderately elongated and slightly curved, the breast rather prominent, the neck moderately long and sloped forward, the crest well forward and forming a straight profile with the slender acute beak. Abdominal uncini (fig. 32) are much smaller, with very short bodies slightly curved downward posteriorly, the breast deep and full, neck and crest similar to those of the thoracic uncini, but the beak much shorter and smaller. The pick-shaped or pennoned setæ (fig. 33) have slightly curved stems, elongated flattened heads, very difficult to see clearly, and high compressed hoods drawn out at a wide angle with the stem into delicate pennants.

No color remains except on the branchiæ, the ventral ones of which have 6 or 7 rich wine-color spots on the barbs and slightly on the stems. On some of the dorsal branchiæ these spots coalesce so that they are chiefly colored, with a few irregular white spots at the base and a more extensive white region toward the tip.

The tube is dark colored, rough, brittle and covered with sand.

This species is known from the type only, taken June 7, at station 4,230, in the vicinity of Naka Bay, Behm Canal, on a rocky bottom, in 108 to 240 fathoms.

EXPLANATION OF PLATE XXXVII.

(All figures are magnified 250 diameters.)

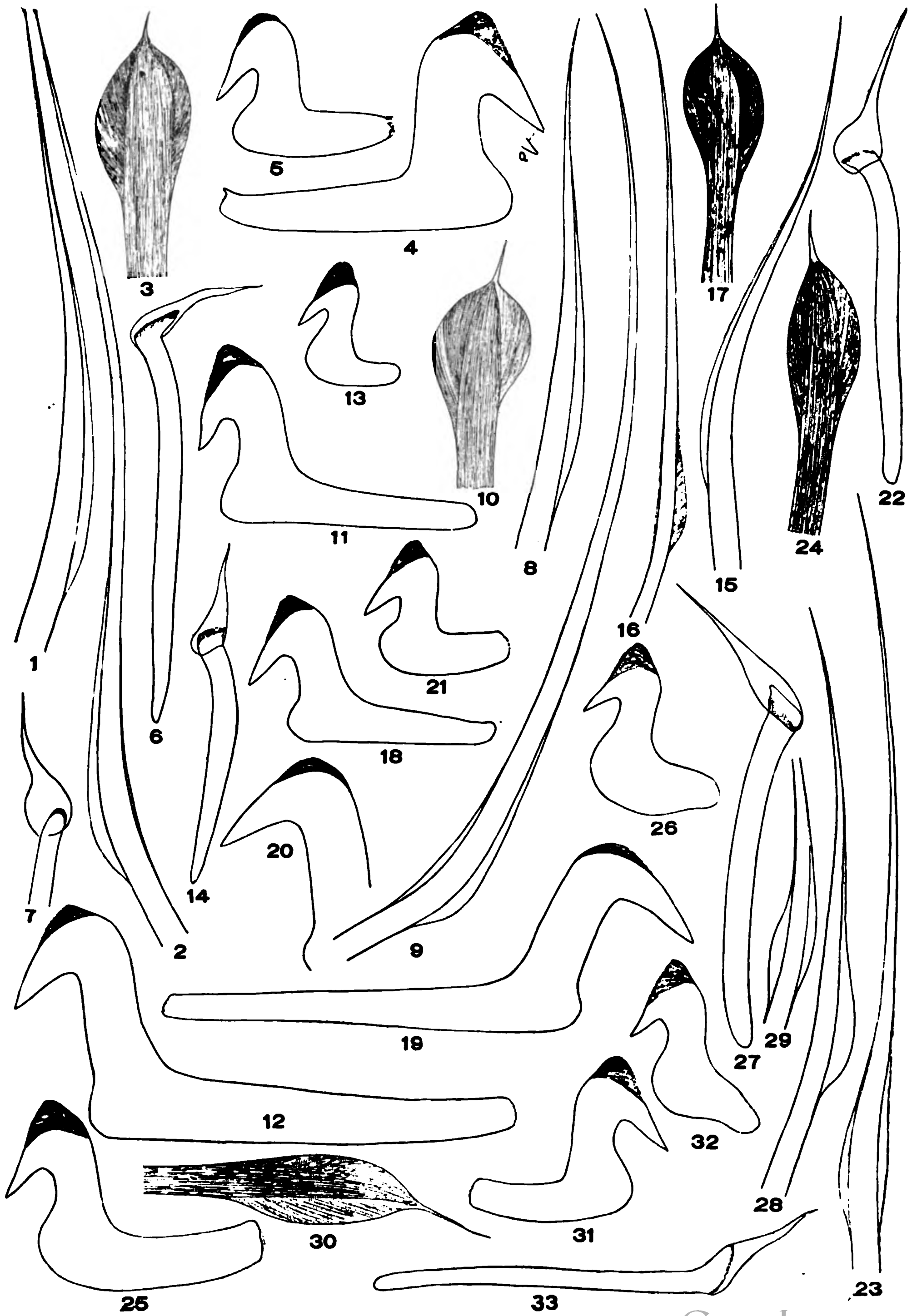
Pseudopotamilla brevibranchiata, figs. 1 to 7.
Fig. 1.—Dorsal thoracic capillary seta from somite VI.
Fig. 2.—Long abdominal capillary seta from somite XX.
Fig. 3.—Spatulate thoracic seta from somite VI.
Fig. 4.—Thoracic avicular uncinus from somite VI.
Fig. 5.—Abdominal avicular uncinus from somite XX.
Fig. 6.—Pick-shaped seta from somite VI.
Fig. 7.—Another view of the end of the same.

Pseudopotamilla occelata, figs. 8 to 14.
Fig. 8.—Dorsal thoracic capillary seta from VI.
Fig. 9.—Long abdominal capillary seta from XX.
Fig. 10.—Spatulate thoracic seta from VI.
Fig. 11.—Thoracic avicular uncinus from VI.
Fig. 12.—Thoracic avicular uncinus from IX.
Fig. 13.—Abdominal avicular uncinus from XX.
Fig. 14.—Pick-shaped uncinus from VI.

Pseudopotamilla intermedia, figs. 15 to 22.
Fig. 15.—Dorsal thoracic capillary seta from VI.
Fig. 16.—Short abdominal capillary seta from XX.
Fig. 17.—Spatulate thoracic seta from VI.
Fig. 18.—Thoracic avicular uncinus from VI.
Fig. 19.—A typical avicular uncinus from the last thoracic somite (X).
Fig. 20.—Portion of another from the same torus showing a larger crest.
Fig. 21.—Abdominal avicular uncinus from XII.
Fig. 22.—Pick-shaped seta from somite VII.

Pseudopotamilla splendida, figs. 23 to 27.
Fig. 23.—Long abdominal capillary seta from XXI.
Fig. 24.—Spatulate thoracic seta from VI.
Fig. 25.—Thoracic avicular uncinus from VI.
Fig. 26.—Abdominal avicular uncinus from XX.
Fig. 27.—Pick-shaped seta from VI.

Pseudopotamilla anoculata, figs. 28 to 33.
Fig. 28.—A shorter abdominal capillary seta from XXVI.
Fig. 29.—Face view of the bladed portion of the same.
Fig. 30.—Spatulate thoracic seta from VI.
Fig. 31.—Thoracic avicular uncinus from VI.
Fig. 32.—Abdominal avicular uncinus from XXVI.
Fig. 33.—Pick-shaped seta from VI.



MOORE. PSEUDOPOTAMILLA.

M 9011

STRUCTURE AND DEVELOPMENT OF THE COMPOUND EYE OF THE HONEY BEE.

BY EVERETT FRANKLIN PHILLIPS, PH.D.,
Harrison Fellow for Research in Zoology, University of Pennsylvania.

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I.—INTRODUCTION.

The morphology of the compound eye has puzzled zoologists for years, and much work has been done on the subject, but so diverse are the views held by the various investigators in the field that we are far from a final solution of the problem. With a view to adding some evidence from the embryological point of view this work was begun, in the belief that a detailed examination of this one insect eye would throw some light on the adult morphology.

The eye of the common honey bee, *Apis mellifera*, is particularly favorable for embryological work, since its growth is gradual and the steps of development well marked out. The material is also easily obtained, and the various stages of growth can be distinguished by the external appearance of the larvæ and pupæ. It is also favorable for a comparison with the development of the eye of *Vespa*, which was described by Patten, since it is desirable to find how far his results can be verified on a closely related form. The large number of ommatidia in each eye make the preparation of sections an easier matter, since it was not necessary to cut so many eyes.

The adult ommatidium of the bee was briefly described and figured by Grenacher in his celebrated work, *Sehorgan der Arthropoden* (1879), and has been figured in works on apiculture, but has never been fully worked out in the adult condition, and no work has been done on the development of the eye. Bütschli (1860) in his work on the embryology of the bee discusses the formation of the eye, but does not go into the subject of the development of the ommatidium.

This work was taken up with a view to getting, first of all, a complete description of the development and structure, and in addition to get some light on certain problems which are of especial interest from a theoretical standpoint. The innervation of the ommatidium, the method of formation and fundamental plan of the ommatidium, the method of modification of numerical plan and structure in the evolution, the arrangement of ommatidia, the homology of various cells in different ommatidia, and the comparison of ommatidia with other sense-organs are questions which have been much discussed, and in this work an effort has been made to apply the observations made to the solution of these problems. This is done not without the realization that some of these things can be settled only from wide comparisons, but with the thought that a piece of work which takes in the whole course of development is of more value than superficial observations of a large number of forms. Some of the theories are merely matters of interpretation rather than of direct observation, and must remain so until decisive observations are made, but in matters of this kind the accumulation of evidence is of decided value.

The formation of the optic lobes and the course of the nervous elements through them are problems which have not been taken up for investigation in this work. Kenyon has worked out the structure of the optic lobes for *Apis* in detail with nerve methods. The technique used in the present work not being suitable for the tracing of nerves, only on matters concerning the nerve endings of the retinula has any investigation been made in this work, and that was not done by Kenyon.

In the matter of nomenclature an effort has been made to avoid the use of new names or of some of the names which have been proposed by some workers who have special theories to uphold, such as calyx, lentigen, corneagen, etc. In the case of the cells which surround the cone I have used the name corneal pigment cells, since they have a double function. In other cases I have used generally accepted names.

The plan followed in this paper is to give, first, a brief description of the adult eye, so that further discussion will be more intelligible, and then to take up the development of the entire eye and ommatidium, followed by a detailed description of the adult conditions, since that was the plan followed during investigation; and is, perhaps, the order which will be most clear to the reader.

This work was taken up at the suggestion of Dr. Thos. H. Montgomery, Jr., now Professor of Zoology in the University of Texas, and was completed under the supervision of Professor E. G. Conklin. To both I am indebted for many valuable suggestions and for help throughout the work.

II.—METHODS.

Larvæ and pupæ were fixed in Flemming's fluid, Hermann's fluid, picro-sulphuric, picro-acetic and picric acid saturated in 50 per cent. alcohol, but of these the Flemming and Hermann preparations yielded the best results. For the smaller larvæ it was not necessary to dissect before fixation, but for older larvæ and pupæ the head was removed to make penetration easier. For adult material, where penetration is difficult, the best fixative was acetic acid, generally a 10 per cent. or 20 per cent. acetic solution in 80 per cent. to 100 per cent. alcohol. Kleinenberg's picro-sulphuric and picric acid in 50 per cent. alcohol were also used with fair results when the head was cut in two.

The material was all cut in paraffine, and it was found that for adult material long embedding was necessary, four to eight hours, to get the paraffine all through the tissues. Some material was embedded for a shorter time to see whether the heat had produced any artifacts in the other material which was embedded for the longer period, but in such cases the lens invariably separated from the retinular layer; no difference was observed in the internal tissues due to long heating.

In staining, the best results were obtained in the use of Heidenhain's iron hæmatoxylin, with the use of a strong mordant for a long time. For material of this kind there seems to be no better stain. It was found that by destaining to different degrees the various parts of the eye would show differences in color, the rhabdome, for example, staining an intense black in rather deeply stained material. The nerve fibrils of the retinula cells also stained black with this stain. Other stains, such as Delafield's hæmatoxylin and eosine or Bordeaux red, were employed with very good results.

For depigmenting Grenacher's solution with a somewhat greater per cent. of acid was used. Parker's solution was also used, though the former gave the better results.

III.—ADULT FORM OF EYE AND OMMATIDIUM.

Before taking up the embryonic development of the eye, it may be well to give a short description of the adult form so that what follows concerning the embryology may be more clear.

The compound eye is made up of great numbers of similar ommatidia surrounded by pigment cells. Among the pigment cells lying between the ommatidia are large numbers of hair cells which secrete the long unbranched hairs which cover the surface of the eye. The entire eye is covered by a layer of chitin, continuous with the chitin of the body wall.

Each ommatidium is composed of an external lens, a crystalline cone and a rhabdome surrounded by eight or occasionally nine long retinular cells which extend from the cone to the basement membrane. Besides these are two pigment cells, the corneal pigment cells, which surround the crystalline cone and about twelve which run the entire length of the ommatidium from the lens to the basement membrane, making in all about twenty-eight cells to each ommatidium. It might be well to include in this enumeration a retinular ganglion cell, since there is probably one to each ommatidium. The lens is chitinous and quite thick and complicated in structure, as will be shown later. The crystalline cone is composed of four highly modified cells, whose nuclei have almost entirely disappeared, and the cells are modified internally by the accumulation of an intercellular secretion which is more dense than the original cytoplasm, but not so dense as the lens. The retinular cells begin at the apex of the cone, and from their proximal ends at the basement membrane send in neural fibres to the retinular ganglion. Each of the retinular cells has decome differentiated on its inner face to form a part of the rhabdome which extends from the apex of the cone to the distal side of the basement membrane. This rhabdome is not chitinous, but is perfectly transparent and slightly more dense than the surrounding cytoplasm. The corneal pigment cells and the outer pigment cells which surround the entire ommatidium serve to prevent the reflection of light in the ommatidium and the passage of rays of light from one ommatidium to another.

IV.—EMBRYOLOGY AND STRUCTURE IN DETAIL.

1. *The Entire Eye.*

The eye arises as a thickening of the hypodermis on the side of the head just posterior to the place where the antennæ arise and just over the optic ganglia. This thickening takes place before the larva is hatched from the egg and the newly hatched larva shows it clearly; the boundaries of the eye are not as well marked as they become by the time the larva is one day old. The optic ganglia are formed at this time, as they arise very early in the development, but the retinular ganglia are not formed until a later larval period.

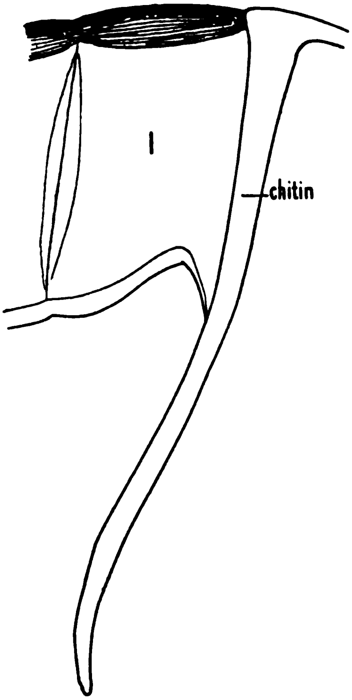

Fig. 1.—Edge of entire eye, showing the relation of lens to the chitin of the body and the strip of chitin running down into the head around the eye.

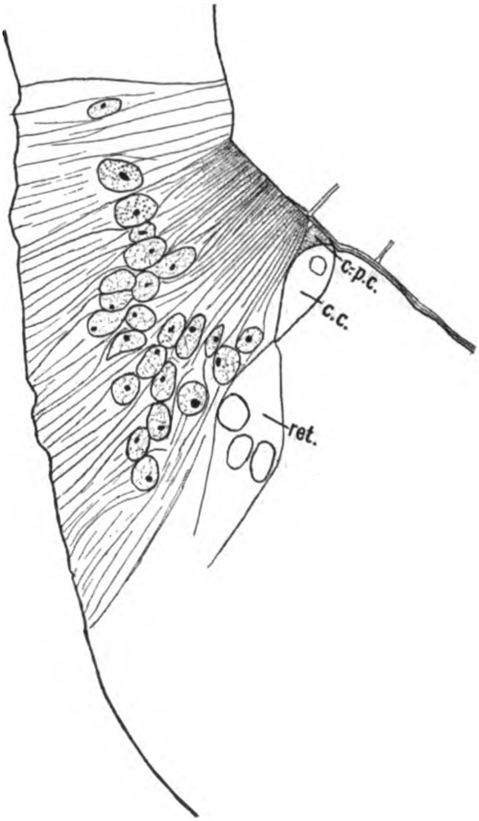

Fig. 2.—Section through edge of pupal eye before infolding occurs, showing transition from hypodermis to eye structures.

This thickened layer of the hypodermis of the newly hatched larva consists of columnar cells with their nuclei arranged at different levels, giving the eye a pseudo-striated appearance. The cells at this time all extend the same distance, so that the eye arises from a strictly one-layered condition. At this time there is no connection with the optic ganglia. During the larval growth the eye increases greatly in size and mitotic figures are abundant, the mitosis always dividing the cells lengthwise, so that the one-layered condition is retained until the close of the larval period.

During the semipupa stage, after the larva is sealed up by the workers of the hive but before it assumes the true pupa form, the one-layered epithelium gives place to a condition in which all the cells do not extend all the way from the outer surface to the basement membrane. This is brought about by the lengthening of some cells, the shortening of others and by the rearrangement of the cells in a manner to be described later. By the time the head has attained the size and shape of the adult, the cells have arranged themselves so that the ommatidia are completely formed and no more mitoses occur. The development of the ommatidia from now on consists of the differentiation of the cell elements until they assume their adult form. The development of the eye as a whole consists of a thickening of the organ and the laying down of a chitinous lens over the surface.

At the sides of the eye of the young pupa the appearance is as shown in text fig. 2, and the cells which correspond to the corneal pigment cells around the ommatidia are quite numerous and shade off gradually into the cells of the hypodermis over the rest of the head. As the eye increases in thickness by the lengthening of the ommatidia there appears a dipping in of the cells of the border, so that there is an invagination all around the eye where the secreting surface of the hypodermis is pulled down. This is shown by a thin sheet of chitin which runs around the eye (seen in section, text fig. 1) in the late pupa and adult eye. This chitin is similar to the chitin of the body proper, but not like that over the eye. This invagination must not be confused with such an invagination as is described by Patten for the formation of the lens layer, for the ommatidia are here completely formed and the corneal pigment cells have moved to their place at the proximal end of the cone before the dipping of the cells here described takes place.

In the formation of the optic ganglia, which takes place by the invagination of cells of the hypodermis, there is formed a brain sheath —a sheath of cells covering the ganglia and still continuous with the hypodermis at the edge of the eye. This layer of cells runs along proximal to the basement membrane and very close to it in the pupa stage. As the retinular ganglia take on their final shape these cells are pushed away from the basement membrane, and are seen in the adult eye as strands of cytoplasm woven in among the nerve fibres between the basement membrane and the retinular ganglion. The nuclei of these cells are smaller and are easily distinguishable from the retinular ganglion nuclei which lie near them (see text fig. 3). On the edge of the nerve bundle this layer is continuous with the brain sheath in the adult. The strands of protoplasm of which this layer of cells is composed after it is perforated by the nerve fibres often run up close to the basement membrane and might easily be mistaken for nerve fibres to the outer pigment cells, but their origin indicates that they are not nerves and there is no indication of any nervous connection for the pigment cells.

Kenyon recognized this layer of cells, which he describes as follows:[1] "The outer mass (first fibrillar mass) presents a lunar appearance in frontal sections (see fig. 1 of this paper), and lies close inside the basement membrane of the retina, being separated from it by sufficient space for the entrance of large tracheal sacs and a thin layer of cells commingled with the fibres from the retina." It will be seen that working with nerve methods this author did not recognize them as nerve fibres, nor did he describe any nervous connection with the pigment cells. Frequently these strands of protoplasm run close to the basement membrane and there spread out as a pyramidal protoplasmic mass lying between the nerve fibrils. This is particularly noticeable in pupa stages before this layer of cells is so greatly distorted.

The basement membrane is made up of a fusion of the proximal ends of the outer pigment cells with the pigmented portion of the retinular cells. This makes a sheet of cytoplasm, perforated where the nerve fibres pass from the retinular cells, which can easily be macerated away from the other elements of the eye and is easily distinguishable on account of its deeply pigmented condition. The nerve fibres from the retina pass through this and are seen as more or less separated on a section through that region (fig. 18). This basement membrane is continuous with the basement membrane of the hypodermal cells. Fig. 10 shows diagrammatically the structure of the base of an ommatidium and the elements which compose the basement membrane, but does not show the separation of nerve fibrils, since that is seen clearly only in cross sections through that region.

There are no tracheæ distal to the basement membrane in the compound eye of the bee such as have been described in other eyes, especially among the Diptera. Exception must be taken to the statement of Hickson[2] that "no spirally-marked tracheæ penetrate the optic tract at any part of its course in Hymenoptera." Between the basement membrane and the retinular ganglion tracheæ with spiral markings occur in all specimens examined (see text fig. 3), but the statement of Hickson holds good for all other parts of the optic tract as far as has been observed. Kenyon also mentions the presence of tracheæ in this region.

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[1] P. 369.
[2] P. 223.

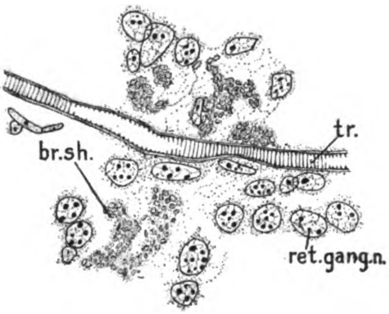

Fig. 3.—Section below basement membrane, showing retinular ganglion cells and nerve fibrils from ommatidia.

2. *Arrangement of Ommatidia.*

The facets of the lens are arranged in hexagons, as is true for so many insect eyes, but this is probably not a primitive condition. Hexagonal arrangement is what is produced whenever any circular objects are closely pressed together, just as the cells of the honeycomb are hexagonal, and this undoubtedly explains the shape and arrangement of the facets. Parker (for Crustacea) looks upon unfaceted eyes as primitive, and probably this is true for insects also. We have, however, in the proximal portion of the eye a different arrangement which is perhaps more primitive than the hexagonal method. At any level proximal to the cone cells the ommatidia are arranged in parallel rows, and the nearer we come to the base of the ommatidia the clearer is this arrangement, until on a section at the level of the basement membrane (fig. 18) we see this parallel arrangement very marked. Since here we get a condition in which the ommatidia are not pressed together and therefore are not modified mechanically, it probably represents a more primitive condition than that found in the lens region. In the pupa, even the facets do not have as marked a hexagonal arrangement as they have later, and in the larva we get an arrangement identical with that of the bases of the adult ommatidia.

The numerical plan and shape of the parts of the ommatidium may have something to do with the arrangement. The retinular cells are eight in number, but four of these are wider than those which alternate with them, and as a result a cross-section of the retina is roughly a square. The outer pigment cells are twelve in number when their arrangement is unmodified by hair cells, and this number readily arranges itself into a square with three on a side, or into a hexagon with two on a side. Since the outer pigment cells are simply strands of cytoplasm they readily accommodate themselves to any change of arrangement and are not, as a rule, without some bend, so these cells could scarcely modify an ommatidial plan of arrangement. The basement membrane is considerably smaller in area than the lens chitin, and as a result the room provided for each ommatidium is considerably decreased, so that in contrast with what has been stated, that the ommatidia are not so crowded proximal to the cone, it might be supposed that the converse would be true. However, the fact is that in cross-section a larger proportion of space is occupied by outer pigment cells, the interommatidial spaces, near the base of the ommatidia than near the lens; and since, as above stated, these cells are flexible and not crowded, it scarcely seems to follow that this parallel arrangement is due to crowding.

The hexagonal arrangement is undoubtedly the common plan, at least as far as the lens is concerned, and the tetragonal arrangement may be derived from it as held by Parker, and his arguments for such an origin seem good; but, on the other hand, the hexagonal arrangement could scarcely give rise to the tetragonal unless preceding the hexagonal facets the ommatidia were in squares, so that the secondary crowding would bring about the primitive arrangement again. Taking again the case of the honeycomb, no additional crowding could possibly make the cells square, for the more the circular walls (the primitive cells) are crowded the more truly they become hexagonal. However, if the walls were made of four parts, as is the cone, and if they were fastened at their bases in parallel rows, then additional crowding might cause the lens to lose its circular outline and become square, in which case the hexagonal arrangement of the lens would be lost. It seems probable that the cone determines the arrangement rather than the lens-secreting cells, and Parker's figures of *Gonodactylus* (Parker, 1890, Pl. VIII, fig. 93), in which the tetragonal arrangement is found in the large ommatidia and not in the small ones, lend support to this view.

To sum up, it seems probable that the arrangement of ommatidia, where they are sufficient in number to be said to have any plan at all, is normally the tetragonal plan. If the cones are somewhat compressed, as they generally are on account of the way in which a compound eye is made up, a hexagonal arrangement of the distal parts of the ommatidia results; but if the pressure is sufficient to cause the cone to lose its circular form then it becomes a square, and the facet plan again becomes tetragonal.

3. *Hair Cells.*

The entire lens of the eye of the bee, especially in the younger individuals, is covered with large hairs, unlike those of the rest of the body in being unbranched. These hairs are secreted by large hair-mother cells which lie among the outer pigment cells between the ommatidia, and their development is of interest on account of the presence in them of an intracellular duct and because of their binucleated condition. In the larval eye these hair cells cannot be definitely located, but there are certain large cells with peculiar nuclei

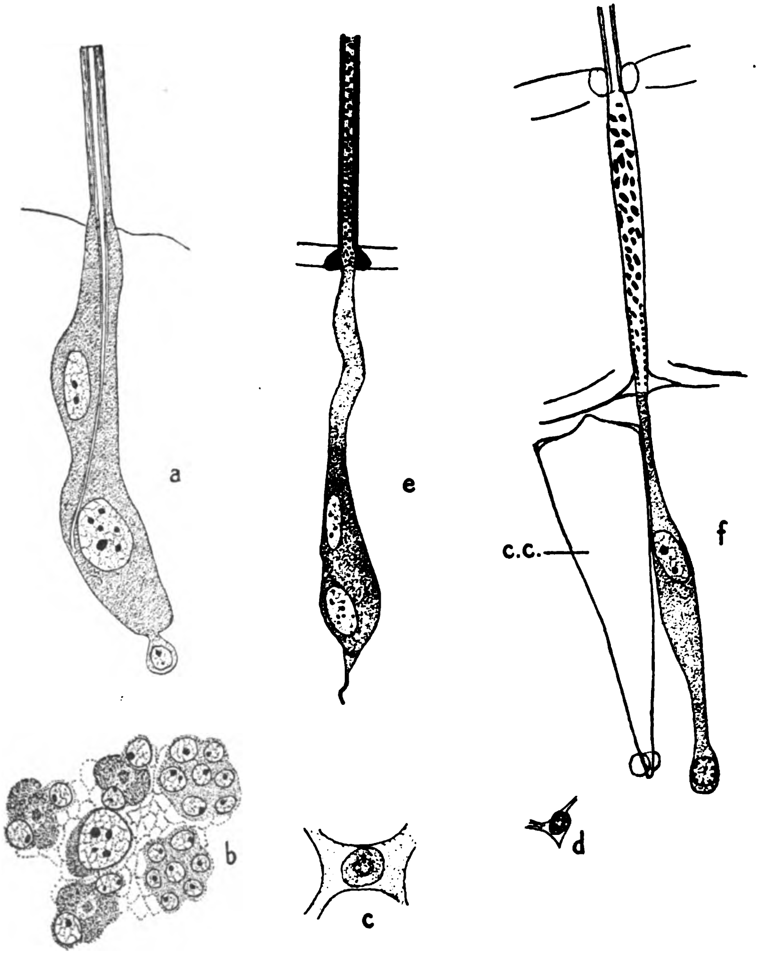

Fig. 4.—*a*. Hair cell of young pupa, showing three nuclei and intracellular duct. *b*. Cross-section through pupal retinulæ, showing one hair cell. *c*. Cross-section through hair just at level of cones, showing structure of intracellular duct. *d*. Cross-section distal to *c* and beyond surface of eye. *e*. Older pupa hair cell. *f*. Hair cell of adult, showing relation to cone and lens.

which are probably hair cells. In the early pupa these cells are large and have two, or sometimes three, nuclei, but when a third nucleus is present it is considerably smaller than the two more distally placed ones. In the early stages this polynucleated cell contains an intracellular duct which opens into the tubular hair, and through this duct passes the secretion products of the cell for the formation of the hair. The hair proper is tubular and in material stained in iron hæmatoxylin darker lines appear in the walls, and these structures extend for a short distance down into the cell proper around the duct. The duct has well-marked boundaries, does not branch, and generally coils around the second nucleus (text fig. 4).

As the lens increases in thickness the hairs elongate by the secretion of the hair cells, and as this goes on the cytoplasm of the cell is used up, until finally, in the adult eye, the cell has about one-sixth the volume it had in the early pupal eye. In the intracellular duct and in the hair duct the products of secretion may be observed in fixed material as darker bodies of irregular shape.

These hairs and hair cells have no nerve connection, as far as I can observe, and are therefore not sensory hairs. Just why the entire eye should be covered by hairs is hard to explain, for they must undoubtedly obscure vision, and since such a hindrance is present we should expect to find it compensated for by some sensory function on the part of the hair. I can find no indication that such is the case. It is worthy of note that the older bees have lost most of the hairs both on the eyes and on the body by the time they need the eyes for prolonged flight. The younger bees, up to nearly three weeks of age, leave the hive but rarely, and then for short distances only, but the older bees which take long journeys have the eyes much more bare. It is also noticeable that all the bees, but especially the drones, brush the hairs so that they all point down toward the mouth just before leaving the hive entrance. No doubt, in the hive, the head, which is so frequently put into the cells, becomes soiled with honey and pollen, and this action of brushing may be merely to remove dirt; but, on the other hand, the arranging of the partly transparent hairs in one direction may produce certain results of refraction which are favorable.

In *Vanessa*, Johansen describes hair cells as running the length of the ommatidia without an intracellular duct and with but one nucleus. He is able to locate these cells at an earlier stage than has been possible for the bee on account of the proximal position of the nucleus. From the figure of a cross-section of the cornea it would appear that these cells are not so abundant as in *Apis*. Patten figures hair cells for

Vespa very similar to those here described, but I am unable to find the nerve connections which he describes. Semper and Breitenbach also describe such hair cells for Lepidoptera.

The number of facets in the different kinds of individuals of the colony differs considerably. The drones (males) have an extremely large number of ommatidia, the eyes meeting on the top of the head, and as a result the three ocelli are crowded down to the front of the head. The workers and queens have a considerably smaller number, about one-third as many, and the ocelli are located at the top of the head. It is not clear why the drones should have a larger number of ommatidia than the females of the colony, since they do not seem to need so much larger range of vision. The only reason which might be suggested from a knowledge of the habits of the two sexes is that at the time when the queen takes her "mating flight" she flies almost directly upward, after a preliminary circle or two near the hive, and then often flies to some distance from the hive; this manner of flying making more probable a mating with a drone from some other colony than her own. Drones do not, as a rule, fly as high as does the queen, and it would be advantageous to have the eyes extending to the top of the head in order to follow the queen's flight. As soon as a queen starts upward any drones which are flying near at hand start upward after her, the eyes on the top of the head making it possible for them to see her.

To say that this difference has arisen on this account scarcely seems justifiable, for it would seem easier for natural selection, sexual selection, or whatever other factor is potent here, to modify the habits of flight rather than to enlarge an organ so much as in this case. This much may, however, be said with a good deal of surety: two things which would be likely to be acted on by selection in the bee are acuteness and range of vision and the power of flight.

V.—RETINULAR GANGLION.

In the early larval stages the optic ganglia are clearly marked out, but the retinular ganglia are not. The only indication of the retinular ganglia is a number of cells which lie near the basement membrane of the eye, principally at the posterior margin. During the larval growth the nerve fibres from the ommatidia grow in from the retinular cells, and as this growth goes on the cells of what are to be the retinular ganglia are pushed farther away from the basement membrane and assume their more definite position. Finally, in the adult animal the nerve fibres from the ommatidia form a relatively compact mass and the retinular ganglion cells are scattered through the fibres in such a way as to have the appearance of a definite ganglion. The nuclei of the retinular ganglion are no longer nearly in one plane, but are scattered for a considerable distance between the basement membrane and the outer fibrillar mass due to the crowding of the nerve fibres.

The question naturally arises as to the number of cells of the retinular ganglion as compared with the number of ommatidia. A count is, of course, impossible, but careful examination reveals that there cannot be many more than one to an ommatidium, certainly not one to each retinular cell. The eight nerve fibres from each group of retinular cells are entirely separate, but lie close together, so that probably one and only one retinular ganglion cell receives the impulse carried from the retina on eight nerve processes, and consecutive cross-sections indicate that the eight nerve fibrils surround the thick part of the retinular ganglion cell where the nucleus is located and transmit the impulse by contact.

In his description of this region Kenyon says:[3] "The elements from the retina terminate each in a small tuft of fine branches in the outer fibrillar body, and come in contact with the fine lateral branchlets given off in the same region by fibres originating from the cells of Berger's granular layer (retinular ganglion)." The tuft of fine branches here mentioned are the separate nerve fibres from the retinulæ. I have been unable to see the fine branches of the retinular ganglion cells.

The retinular ganglion cell in turn sends in its fibre through the first fibrillar mass, and then through the outer chiasma to the opposite side of the group of ganglia, where the impulse is given over to a cell of the first optic ganglion. From here on the tracing of the fibres requires special nerve methods which were not employed in this work. However, this much is evident: the cells of the first optic ganglion send their fibres through the second fibrillar mass and through the inner chiasma to the second optic ganglion, where the impulse is probably again transferred to another cell which, in turn, carries it to the brain. The course of these fibres has been worked out in detail by Kenyon (1897), and in my work I find nothing to contradict his results, although the methods used in my work were not such as to warrant either a positive denial or confirmation of his work.

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[3] P. 374.

VI.—The Ommatidium.

1. *The Larva.*

In the larva, just after being hatched from the egg, I have been unable to find any indication of the grouping of cells which are later to go together to form a single ommatidium. The eye at this time is a simple layer of the thickened hypodermis with the nuclei arranged one above the other. At this time, and throughout the entire larval period, mitotic figures are abundant, the spindles always having their axes at right angles to the length of the cell and dividing the cells lengthwise. The division figures seem to be more abundant near the outer surface of the epithelium.

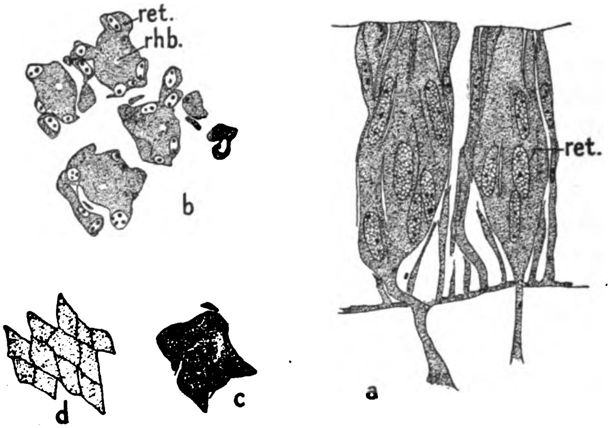

Fig. 5.—*a.* Longitudinal section of larval ommatidia. *b.* Cross-section near surface of eye, showing first differentiation of rhabdome (rhb.) as a clear space in the retinula (ret.). *c.* Cross-section at a lower level. *d.* Cross-section of a very young larva, each division line representing a complete ommatidium.

About one day after leaving the egg, when the larva has about doubled in size, a tangental section of the eye at right angles to the long axes of the cells at the outer surface reveals a grouping of cells as represented in text fig. 5*d*. The lines in this figure do not represent cell boundaries but are the boundaries of groups of cells; each group contains four or five cells at this time, the nuclei of these cells being directly one above the other. The cell groups are tetragonal and are arranged roughly in parallel rows. In longitudinal section these groups appear as made up of long strands with superimposed nuclei about the diameter of the entire width of the group of cells. That these are the beginnings of the ommatidia is evident since they can be traced through all the larval stages to the pupa, where the ommatidia are definitely marked. This is further indicated by the fact that they are arranged in the same way as are the proximal ends of the ommitidia, even in the adult eye. It should be borne in mind that this epithelium is strictly one-layered, and this is true all through the larval period.

During the larval period, as above stated, mitotic figures are abundant, and as a result of these divisions the groups come to be composed of more and more cells, but it is not until a late larval period (about four and a half days from the hatching of the egg for worker larvæ) that any further differentiation is observable, except possibly that the nuclei of some of the cells are larger than others in the same group. At this late larval period the cells arrange themselves as a spindle-shaped mass surrounded by smaller cells whose smaller nuclei lie in the space left at the outer end of the spindle. Mitotic figures are now absent except an occasional one in the smaller cells, but so far none have been observed in the larger centrally placed cells of the group. The number of cells in the spindle is hard to determine, since the nuclei are at different levels and the cell boundaries are not visible. All the nuclei of the central bundle of cells are some distance below the surface. There are certainly, however, not more than eight or nine, the number of retinular cells of the adult ommatidium. At the distal end of this spindle a differentiation of cytoplasm takes place, and a clear space is formed in the centre of the cells in the very granular protoplasm, and this I believe to be the beginning of the rhabdome. A cross-section near the outer surface of the cell mass shows this clear space surrounded by granular cytoplasm of the spindle cells, and this in turn surrounded by nuclei arranged around the central bundle. These outer nuclei are not as yet differentiated, so that their future fate cannot be determined. The cells of the spindle by this time have sent out protoplasmic processes toward the optic lobes which become the nerve fibres of the ommatidium, so that at any rate some of the spindle becomes the retinula.

Several facts seem to indicate that the spindle-shaped centre of the ommatidium goes to form only the retinula: (1) There are no nuclei near the outer surface, as one would expect were crystalline cone cells to be formed from any of the cells; (2) there are not enough cells to form both retinula and crystalline cone cells, and since no mitotic figures have been observed they have undoubtedly ceased division; (3) a clear space is formed at the distal end of the spindle by a differentiation of the cytoplasm, possibly the beginning of the rhabdome, since it is in this portion of the retinula that the rhabdome is seen in the youngest pupal eye observed (just after the semipupa stage). The number of nuclei around the spindle throws no light on this, since they are still dividing occasionally and their number in the adult is not fixed.

Considerable stress has been laid on the fate of this spindle-shaped mass of cells, since the determination of this fact alone is of such great importance in the consideration of the morphology of the ommatidium. That the outer pigment cells are morphologically peripheral to the crystalline cone and retinula no one would deny. The position of the corneal pigment cells might be a doubtful point if they were derived from a separate layer of cells formed by invagination of the entire eye, but as no such invagination occurs in the bee, and as at an early pupa stage they are clearly outside the cone, I think there can be no doubt as to their morphological position. The question as to the relative morphological position of the crystalline cone cells and the retinular cells is, however, not so clear.

According to Grenacher the ommatidium is two-layered, and the lens and cone are morphologically distinct from the retina. If this view is held, then the question stated above does not exist; but such an interpretation can no longer be held on comparative anatomical or embryological grounds, as has been shown so well by numerous investigators, the evidence for which it is not necessary to give here. Suffice it to say that, as has been shown previously, the ommatidium of *Apis* arises from a one-layered epithelium, and all the cells are morphologically equivalent. Taking into consideration, then, only such views as are based on such interpretations, we find two opposing theories.

According to Patten, Kingsley and others, the crystalline cone is sometimes continuous with the rhabdome; these two would therefore be the morphological centre of the ommatidium, while the retinula must arise from cells outside this. When the crystalline cone is not continuous with the rhabdome, Patten still considers the cone as the centre, since he describes processes running from each cone cell around the rhabdome but inside the retinula (as in *Vespa*). To this interpretation those investigators who consider the crystalline cone as the terminus of the nerve fibres would probably agree. On the other hand, Watase holds that the ommatidium is a morphological invagination of which the retinula is the centre, and the cone cells, lens cells (homologous with the corneal pigment cells of *Apis*) and pigment cells follow in the order named. By this interpretation the rhabdome, cone substance and lens are homodynamous. These two views seem in no way reconcilable, and more investigation is necessary to decide between them, since it scarcely seems probable that both plans exist, since all ommatidia are probably the result of one kind of development.

Patten bases his view on the fact that the cone cells are continuous with and part of the rhabdome, but surely in *Apis* there is no such continuity, since all through the development they are separate, and in the adult eye there is a sharp line of demarcation between them, and they also react very differently to stains. In *Vespa*, Patten admits that the rhabdome is not continuous with the crystalline cone cells, but in this case he describes processes between the rhabdome and retinula which correspond to the processes which form the rhabdome in other forms. Since, as will be discussed later, the rhabdome is really part of the retinula, being formed as an intracellular secretion, any such process from the cone cells would have to pierce the retinula cells to occupy such a position. No such processes occur in *Apis*. If such a view be held because it is necessary in some way for the nerve fibres to reach the crystalline cone, on the assumption that the nerves end there, such a necessity disappears, for, as will be shown under a discussion of the innervation of the ommatidium, the cone is in no way a nerve terminus. Such a theory of innervation does not seem justified for any ommatidium, and therefore the necessity for this conception of the morphology disappears.

On the other hand Watase based his view largely on the eye of *Limulus*. This view commends itself on account of its extreme simplicity, since all ommatidia readily lend themselves to the plan of diagrammatic representation used by Watase with this interpretation. Watase seems to have advanced this theory rather for the purpose of giving some explanation for the existence of the rhabdome than for the morphology of the entire ommatidium. There is, I think, no reason to believe that the rhabdome was ever a chitinous substance, and in that sense it is not homologous with the lens. In the ommatidium, as we now know it, the rhabdome is an intracellular secretion full of nerve fibrils, and is far from being a hard chitinous growth. To that extent, then, Watase's conception seems an error. If, however, we look on the lens, cone substance and rhabdome as secretions (non-living protoplasmic differentiations), of which the lens only is an extra-cellular secretion, then the homology may hold. Acording to this view, then, the ommatidium did not arise as a pit filled with chitin, but rather the sinking in of certain cells, with a corresponding retention of the secretion inside the cell, has taken place with the assumption of new functions. Parker has argued that the retinular cells cannot be considered as homologous with the lens secreting cells, since the lens cells secrete on their distal surface while the retinular cells secrete on their lateral surfaces. My observations show that both cone cells and retinular cells form their secretions intracellularly and from their very positions they could not secrete on their distal surfaces, but this does not seem to me to be any objection to the theory of Watase, since in the invagination of the cells and the taking on of new functions new forms of metabolic activity might easily be acquired.

Since, however, in the embryonic development of the ommatidium of the bee we find a stage in which the retinula is formed without cone cells on the distal end and with the rhabdome partly formed, the only inference, it seems to me, is that the cone arises from lateral cells, and the corneal and outer pigment cells are, of course, still more peripheral. From this, then, it seems to follow that the conception of Watase concerning the morphology of the ommatidium is the correct one. There is, so far as has been observed, no real invagination, but such a thing would scarcely be expected in so compact an organ; neither have I observed the actual overgrowing of the cone cells, but the conclusion seems inevitable that the retinula is the centre of the ommatidium.

Some compound eyes have been described in which, in the adult eye, the retinular cells extend outside the cone to the lens. Such cases are found when the number of pigment cells is reduced or when they are entirely wanting, and it is safe to assume that the distal lengthening has taken place secondarily, late in development. From the migration of the corneal pigment cells of the ommatidium of the bee, to be described later, we see that a late rearrangement is possible, and it seems more plausible to assume that such cases are a secondary modification rather than that there are two ground plans of ommatidia, one of which has its retinula centrally placed, the other has the cone cells inside the retinula as the axis.

The retinular spindle of the larva resembles in appearance various sense buds throughout the animal kingdom, such as taste buds and lateral line organs of vertebrates, the æsthetes of Chitons, etc. These sense buds often have some marked differentiation of the cytoplasm internally to enable the peripheral organ to perform its function. This similarity is more than superficial, however, for the method of innervation which will be described in detail later is from the sense cell toward the central nervous system, and this is the method for many of these sense buds, although the opposite direction of fibres is described for some (*e.g.*, taste buds).

It is safe to assume that these sense buds are accumulations of single sensory cells, such as are widely known (*e.g.*, sensory epithelial cells of *Lumbricus*, epithelial sensory cells [Flemming's cells] of Molluscs), giving greater efficiency at a certain spot, and that the internal differentiations are but secretions or cytoplasmic differentiations due to the specialized condition of the cell. Granting these facts, then, sense buds are homologous of necessity only in their origin from an epidermal tissue, although the homology may be greater. Since sense buds are known which are sensitive to touch, taste, smell, sight and vibration waves, it seems entirely unnecessary to assume that a light-perceiving organ, such as an ommatidium, has arisen as a modification of some other kind of sense bud, rather than that it arose as an accumulation of epithelial cells already sensitive to light.

Since we know that single cells are acted upon by light waves (*e.g.*, Protozoa), and that epidermal cells often give rise to nervous impulses when acted upon by light (*e.g.*, skin of the earth worm), there seems no reason for assuming that the ommatidium has arisen other than by an accumulation of such sensitive cells and then by invagination a light-refracting organ has been formed over it. Such a view is directly opposed to the view of Patten that the ommatidium is a hair-bearing sense organ. As will be shown later, his theory is untenable on account of the absence of the essential structure for such a homology—the hair. There is not only no indication of such an organ for the eye, but no need for such a complicated theory of the origin of these organs, since easy transition steps from a single cell sensitive to light to the ommatidium are obtainable and such an origin seems far more probable.

Johansen (1893), in his description of the development of the eye of *Vanessa urticæ* L., figures and describes a spindle-shaped mass of cells which is the ommatidium of the pupa when two days and one hour old. He has also observed the same spindle mass in the young pupa of *Sphinx euphorbiæ*. This differs from what I have described for *Apis* in that the corneal pigment cells and cone cells lie distal to the retinula, and I am led to conclude that he has observed a stage just after the sinking in of the retinula, a stage which I am unable to describe for *Apis*. At any rate his conception of the morphology agrees with mine, since the retinula is in the centre of the ommatidium and the cone cells and corneal pigment cells are lateral to it.

2. *Pupa.*

During the so-called semi-pupa stage, just after the larva is sealed up by the workers of the hive, and before the bee is a complete pupa, very rapid growth takes place, and the eye increases still more in size and becomes more and more differentiated until at the beginning of the pupa stage proper the ommatidia are completely formed. The exact method by which this differentiation takes place is difficult to learn, since the growth at this time is so very rapid that it is practically impossible to get all the stages. The head of the insect grows very rapidly and the eyes keep pace with it. The retinular cells become longer and broader, and the retinulæ lie closer together. The cone and corneal pigment cells come to lie at the distal end of the retinula by the method previously described. When the pupa stage proper is entered upon, the area of the eye is practically that of the adult eye.

The various stages of the pupa period are easily distinguishable externally, and this fact is of great value in the selection of material. The eye is first white, like the rest of the body, then pink, then brown, and finally, as the other parts of the body take on their adult colors, black. These changes of color are due to the deposition of pigment in the various cells of the ommatidium, pigment in the corneal pigment cells being red in color, giving the first color externally, and the darker pigments of the other cells obscuring this color at a later period. These changes enable one to choose the desired material by simply uncapping the cells containing pupæ without removing the bee from its cell, since the head is always toward the outside.

From this stage on it becomes necessary to discuss the various parts of the ommatidium separately. Such a method tends to give the impression of a lack of continuity in mode and time of development, but the drawings which accompany the description are made of the entire ommatidium, and these will show the relative size and degree of development at various stages. The order followed is from the retinula to the more lateral cells.

a. The Retinula.—The retinula cells are eight in number normally, but numerous ommatidia are observed in which nine cells are present. In the earliest pupa stage (fig. 3) these cells extend from the proximal end (apex) of the cone cells to the basement membrane, and each cell has a protoplasmic process extending through the openings in the basement membrane toward the optic lobes, which later functions as the nervous connection of these cells with the cells of the retinular ganglion. At this time the only indication of the rhabdome is the clear space at the distal end which was described for the larval ommatidium; its differentiation has gone on little, if any, during the rearrangement of cells. The cytoplasm at the distal end of the cells is more granular than elsewhere, and by the time the eye has reached the stage figured pigment is laid down around the forming rhabdome. This is the first pigment laid down in the ommatidium, but at almost the same time the corneal pigment cells acquire pigment. The spindle shape of the retinula so marked in the larval condition is still retained, the retinula being widest at about one-third of the distance from the cone cells to the basement membrane. The relatively large nuclei of the retinula at this time are near together, and in no definite arrangement in the thickest portion of the cell group. The cytoplasm of the cells is uniform except as described for the distal end, and the cell membranes between the various cells are not visible. The outside boundaries of the retinula group at this time and all through development mark off the retinula from its surrounding pigment cells very sharply, and the difference in the appearance of the protoplasm makes it impossible to confuse the various cells.

The portion of the retinula which lies between its thickest part and the basement membrane is a strand of protoplasm circular in cross-section and without any signs of differentiation. As the basement membrane changes its position, by a process to be described later, coming to lie near the optic ganglion, this portion of the retinula becomes longer, and the changes which take place in the retina consist of the making over of this strand of protoplasm into the retinula cells proper. This change progresses proximally and consists in the widening out of the cells with its accompanying rhabdome formation. The nuclei shift as the retinula enlarges and elongates until we reach a condition (fig. 2) in which two of them are at one level and the other six (or seven) are at a lower level and arranged in a rosette.

At the time when the nuclei are arranged in this manner, the most distal portion of the retinula becomes arranged in a definite rosette, caused by each of the cells forming a projection which shows its distinctness from the others in the group in cross-section. This arrangement also progresses proximally until in the adult condition it is found throughout the length of the retinula. At the same time the inner portion of the mass becomes still more differentiated, and in the stage just mentioned the axis of the distal end is occupied by a strand of protoplasm which takes the iron hæmatoxylin stain (the future rhabdome) surrounded by a clearer protoplasm. Outside of this clear area the protoplasm is granular and pigment deposition takes place here, keeping pace with the inner differentiations, and these changes also progress toward the basement membrane. The rhabdome formation precedes slightly the formation of the clear protoplasm around it, and the proximal end of the forming rhabdome shades off gradually into the surrounding undifferentiated cytoplasm.

The nuclei gradually move inward as the cells assume their adult form until they come to rest at about one-third of the distance from the cone to the basement membrane, which on account of the tapering of the retinula is at about the centre of the cell, as far as mass of cytoplasm is concerned. One of the nuclei, however, moves proximally until it lies about half-way between the other nuclei and the basement membrane. Where a nucleus is present, the retinula cell is slightly pressed out, encroaching on the outer pigment cells, and the upper nuclei are not all at the same level. The one nucleus which occupies a more proximal level is separated by some distance from any of the others, however, and, owing to the regularity with which it is found, cannot be considered as due merely to a mechanical shifting. In the older stages of development it becomes difficult to count the nuclei of the retinula since they are at different levels, but I have been unable to see anything which would lead me to suspect that this proximal nucleus was other than one of the retinular nuclei. Neither is there any indication that the presence of this nucleus is accountable for the presence of nine retinular cells in some ommatidia, for it is found in all ommatidia and the nine-celled condition is comparatively rare.

The rhabdome differentiation proceeds until it reaches the distal surface of the basement membrane where it ends abruptly. In the pupa stages I am unable to find the nerve fibres which in the adult eye run parallel with the rhabdome and send fine fibrillæ into it. It will be noticed, however, that in the pupa the rhabdome is wider and not so definite in outline as it is in the adult eye, and the nerve fibrils are no doubt included in this darker central body which I have identified as the rhabdome. Both rhabdome and nerve fibrillæ are but differentiations of the cytoplasm of the retinula cells and their development takes place together. The rhabdome is probably not a uniform structure, but no doubt contains a mass of fibrillæ, the endings of the nerve fibres. I am unable to see any such structures, however.

The development of the retinula consists, then, in the changing of the sense-bud-like spindle of the larval eye into a long column of cells with a clear shaft through the centre, through which light can pass to reach the nerve endings in it. From the previous description it will be evident that the rhabdome is not formed by processes from the cone cells, which are present from the beginning of ommatidial development, but is an intracellular differentiation of the retinula, there being a sharp line of demarcation between the cone cells and rhabdome throughout their development.

b. The Cone Cells.—The cone cells are four in number, and in the early pupa stage (fig. 3) the cone is spindle-shaped and lies directly distal to the retinular spindle. The nuclei are large and spherical, and lie slightly distal to the centre of the cell. The cytoplasm is granular, especially in the distal portion of the spindle, and the cell membranes are well marked.

Very soon the cytoplasm begins to be differentiated, and by the time the pupa has reached the stage figured (fig. 2) vacuoles begin to appear in the proximal end of the spindle, which marks the beginning of the formation of the clear cone substance. The cells now increase in size considerably, and at the same time the number of small vacuoles increases. Later these vacuoles unite, and finally a condition is reached in which the proximal end of each cell is occupied by one large clear vacuole. The cell boundaries remain distinct and a thin layer of granular protoplasm remains surrounding the vacuole, so that it is strictly an internal secretion and not to be interpreted as a secretion poured out on the inner face of each of the cells. This process of differentiation or intracellular secretion goes on until the nuclei, which decrease in size and become long and narrow, are pushed to the distal and lateral portion of the cell, where they remain in the adult eye. These nuclei are filled with fine chromatin granules. The cone in the meantime becomes wide at the distal end, and elongates very much to assume its true cone shape, and all that remains of the original cytoplasm is an extremely thin sheet all around the cone. I am inclined to attribute the descriptions by some authors of nerve fibrils on the cone to the shrinking of this thin film under certain fixatives. There is no nervous connection with the cone, nor does it appear to have any function save transmitting light rays to the sensitive retina.

There is no indication of any prolongation of the cone proximally, either to form the rhabdome, as previously described, or to form protoplasmic processes surrounding the rhabdome inside the retinula cells, such as Patten describes for *Vespa*. Such fibres could not exist unless they were to pierce the retinula cells, since the rhabdome is really a part of the latter; and since the cell boundaries of the cone and retinula are so well marked I feel sure that no such ingrowth occurs.

Equally unsuccessful has been a search for any additions to the cone at the distal end. In his work on the embryology of the eye of *Vespa*, Patten describes a layer of cells distal to the cone which arose by an overfolding of the sides of the entire eye, and which gave rise to the lens. In a later paper (1890) he disposes of his invagination theory, but describes a pouring out of chitin from the distal end of the cone, which secretion he mistook for the layer of nuclei at an earlier time. From my examination of *Apis* material I am unable to find anything which could be mistaken for nuclei in that position (unless it be the corneal pigment cells which are lateral to the distal end of the cone) or for chitinous secretion of the cone; and for this insect eye, at any rate, I am led to doubt the validity of his homology of such a structure with the pseudocone of ommatidia of the "pseudocone type," since the distal end of the cone is perfectly well defined at every stage observed.

The differentiation of the cone consists in a transformation of a cone without any refractive secretion into one in which this secretion fills all the cells proximal to the nuclei, or, in other words, a modification of an acone condition into an eucone condition, to use terms introduced by Grenacher for adult conditions of some eyes. There can be no doubt that this was the course taken during the evolution of the eucone ommatidium. Similarly, Hickson has shown that the so-called pseudocones described for many insect eyes are but instances in which the secretion has accumulated in the distal end of the cone rather than in the proximal end. While the distinction drawn between these three kinds of cones is justifiable, yet there seems nothing to oppose the view that they are but modifications of one primitive type. The acone ommatidia have no clear refractive substance differentiated in the cone cells, and are considered as the primitive type of eye. The pseudocone cones with the differentiation of clear cone substance distal to the nuclei and the eucone cones with a proximal secretion are but modifications of the primitive type.

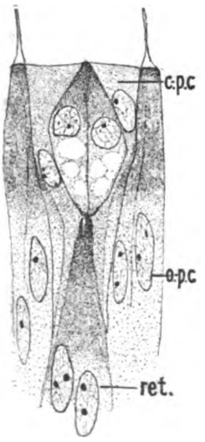

Fig. 6.—Young pupal ommatidium at time of migration of corneal pigment nuclei.

c. The Corneal Pigment Cells and the Lens.—The lens is secreted by the two cells which have been designated corneal pigment cells. In the very earliest pupa stage these cells lie distal and lateral to the cone cells, and since they are thus placed at this time, and since their secretion product is distal to the cone, they are next in order in going out from the axis of the typical ommatidium.

Before these cells begin their secretion, however, the nuclei migrate down the sides of the spindle-shaped cone and come to lie around the apex of the cone. The cause of this migration is probably purely mechanical, viz., the enlargement laterally and distally of the cone; at the same time the nuclei are thus brought nearer to the source of nutriment. As this shifting takes place the nuclei, originally ovoid, become crescent-shaped, and finally almost encircle the apex of the cone. Strands of cytoplasm connect the nucleated portion of the cell with the distal portion, which remains at the point where secretion is to take place. As the cone enlarges and the cell substance of the corneal pigment cells is used up in the secretion of the lens, the portion distal to the cone becomes reduced until in the adult eye it is almost entirely absent.

Almost immediately after pigment is first formed in the retinula cells, it begins to be deposited in these corneal pigment cells. Owing to the fact that the retinula pigment is at first small in quantity, and since there is none in the outer pigment cells at this time, the pigment of these distal pigmented cells, which is red, gives a pink color to the entire eye in the early stages, rather than the brown or black color possessed by the other pigment, as is true in late stages.

The granules of pigment are large and red in color, and when treated with depigmenting mixtures do not disappear, but become somewhat lighter in color.

The lens is secreted by these cells in much the same way as is ordinary chitin over the entire body of the bee. This chitinous covering is deposited in layers which are easily visible in the adult lens. In addition to these cells the outer pigment cells also seem to enter into this. In the pupal eyes before any chitin is deposited by the corneal pigment cells thin sheets of chitin extend out from the outer pigment cells, and since these cells are arranged at their distal ends in a nearly hexagonal manner a cross-section of these plates shows the future boundaries of the facets. In the adult eye the portion of the cornea which directly overlies the outer pigment cells differs slightly from the part directly over the cone in refractive index and in general appearance, so that I think it probable that the space between these sheets of chitin in the larva is filled by a secretion of the outer pigment cells. If this be true, then every cell which enters into the formation of the compound eye has to do with some sort of secretion, either intra- or extracellular.

The structure of the chitin laid down by the corneal pigment cells is not uniform, the outermost layer being more dense than the rest, with a decided tendency to take up an iron hæmatoxylin stain, the middle or main portion being arranged in alternating layers of different density, and the inner portion taking a protoplasmic stain, such as eosine or Bordeaux red.

From this description it will be seen that the corneal pigment cells (Hauptpigmentzellen, pigment cells of the first order) are homologous with the corneal hypodermal cells of the crustacean and apterygote insect eyes. In all crustacean compound eyes small nuclei are described as lying distal to the cone cell nuclei (or Semper's nuclei), and these are the nuclei of the cells which secrete the lens. When the ommatidia are arranged in facets, two such cells are present. In the Apterygota, *e.g.*, *Lepisma saccharinum*, *Orchesella*, etc. (Hesse, 1901), these two cells are present and occupy a similar position or may be placed slightly more laterally. These two cells are characteristic of these two types of compound eyes. On the other hand, the compound eyes of most pterygote insects have the two pigment cells of the first order (corneal pigment cells), and do not have the corneal hypodermal cells. Hesse (1901) concluded that these two kinds of cells are homologous from an examination of adult eyes, and considered his point strengthened by the fact that Johansen had described these pigment cells as being distal to the cone cells at an early stage. Johansen did not describe them as homologous, however, and derived the lens from another source. From an examination of *Apis* I am convinced that Hesse was correct in his deductions, for in this case the cells are not only homologous, but the pigment cells here have identically the same function as have the corneal hypodermal cells of the other eyes.

As mentioned above, Johansen failed to see this homology and describes and figures the lens as being secreted by the cone cells. It has been pointed out with sufficient detail that no such interpretation is tenable for *Apis* at least, and we may well doubt its occurrence in *Vanessa*. In Pl. 23, fig. 11, he figures the secretion of the lens by the cone cells and shows the corneal pigment cells extending to the distal margin, and I am led to conclude that he has overlooked the position of the pigment cell which remains distal to the cone.

d. The Outer Pigment Cells.—These cells from the earliest larva to adult stages extend the entire length of the ommatidium, and are what are known as accessory cells in many eyes. They, like all the other cells of the eye, are of ectodermic origin, there being no cells from the mesoderm in the eye of *Apis*, such as are described in some eyes. These cells are, normally, twelve in number, but when hair cells are present between the ommatidia, which is very frequently the case, this number is increased so that any definite enumeration is impossible; and since these cells serve merely to fill the interommatidial spaces and to prevent reflection inside the lens, no more definite arrangement is required. The nuclei of these cells lie proximal to the cone in pupal stages, but in the lengthening of the cone they come to lie at about its middle.

Pigment is deposited in these cells quite early, but not until after it has appeared in both the retinular and corneal pigment cells, and is most abundant at the two ends of the cell. It will be noticed that of all the cells of the eye which contain pigment none acquire this until they have begun to form the secretion to which they give rise. The rhabdome is the first secretion formed, and pigment first appears in the retinula; later the lens secretion appears, and then pigment appears in the secreting cells, indicating, it seems to me, that this pigment is of the nature of a by-product, although it is of itself of value. From one point of view, pigment itself is a secretion, but the accumulation of pigments often accompanies other secreting activities. Concerning any possible movements of the pigment under different light conditions, no observations have been made.

In the region where the basement membrane is formed these cells are deeply pigmented, and the line of demarcation from the cell below is very marked. At this point, also, and only here, the cells are fused with the retinular elements. This intimate union can exist only when the retinular elements have filled out to that point, since in the pupal stages that portion of the retinula is a thin strand. The retinular cells here are also deeply pigmented.

3. *The Adult Ommatidium.*

In the discussion of the changes which take place during the pupal period many of the details of the adult ommatidia are given, and to avoid unnecessary repetition only such things as have been omitted will be discussed here.

a. The Retinula.—The adult retinular cells are extremely complicated structures, due to the fact that each cell has so many differentiations internally. The central part of each cell is differentiated into a sector of the rhabdome, which is possibly a dead secretion, but of this there is room for some doubt. Outside the rhabdome is an area of clear protoplasm in which the nervous elements of the cell are found, and still outside of this is the granular portion of the cell in which pigment granules are found. Each of these cells then secretes part of the rhabdome, acts as a pigment cell by the accumulation of pigment on its outer surface, and is, in addition, a nerve-ending cell.

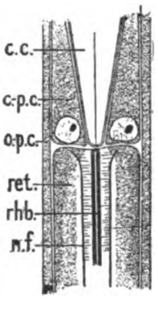

Fig. 7.—Diagram of part of ommatidium, showing apex of cone and distal end of retinula.

The innervation of the ommatidium is a question over which there has been much discussion, and various views have been put forth. The views can, however, be classed into two groups: those which make the cone cells the nerve-ending, and those which find the terminations in the retinula. It has been shown conclusively by numerous investigators that the cone has nothing whatever to do with receiving light stimuli, and it would be useless to take up the arguments against this view, any more than has been done in showing that in the development the cone and rhabdome are separate.

Those who hold that the retinula is the nerve-ending of the ommatidium have not always been able to show in a satisfactory manner just how this innervation takes place. On this point two views have been held: (1) that the retinula is innervated by nerve fibrils from the retinular ganglion which run into the retinular cells or rhabdome, or (2) that the retinular cells are themselves ganglionic epidermal cells which send in nerve fibres to the retinular ganglion. From the description which has preceded it is evident that the second of these views is the one here held for the eye of the bee. Before going into a detailed descripton of the nervous elements in the cells concerned, let us first examine the problem.

In the first place, it seems reasonable to assume that during the course of the evolution of light-perceiving organs the first condition was that in which certain cells of the hypodermis became sensitive to light, or possibly heat, through the accumulation of pigment or some other change in the cytoplasm. Such cells would arise before there were any cells in the central nervous system to receive their nerve stimuli, and it may be assumed without danger that such cells would send in processes to the centrally placed nerve cells, when the time for nerve connections arrived, rather than that the nerves arose from the central nervous system. In other words, the peripheral nervous system is older than the central nervous system which elaborates the impulses, and on hypothetical grounds, a basis which is rather unsafe in zoology unless backed up by observations, we may assume that the innervation is centrad.

From the standpoint of embryology, we find that the eye epidermis is formed and even the ommatidia are differentiated before the retinal ganglion cells have assumed their adult position or are connected with the optic ganglia. Not only that, but the strands of cytoplasm which become the nerves of the ommatidia arise from the retinula cells and grow centrad.

In the adult condition we find that the nerve fibres are continuous with the cytoplasm of the retinula and run to the retinular ganglion, where they surround the nuclei of the ganglionic cells. There is no indication of long nerve processes from the ganglion cells toward the eye.

The nervous elements of the retinular cell proper consist of a differentiated portion of the cytoplasm inside the clear area which lies outside the rhabdome. This nerve fibre can be seen best in sections stained in iron hæmatoxylin, where it stains black. From this fibre, which starts at the distal end of the cell and runs parallel to the rhabdome, smaller fibrils are given off which run into the rhabdome where they all end. More properly speaking, these fibrils are further differentiations of cytoplasm which lies between the main fibril and the centre of the retinula. These fibrils extend from the fibre to the rhabdome along the whole length of the retinula proper, so that the nerve-endings are very numerous. Below the basement membrane these main fibres can be traced as dark lines in the centre of the protoplasmic processes to the retinular ganglion. All of these fibres are best seen on cross-sections where they stand out as black dots, but they can also be seen on longitudinal sections. It is probable that the cause of the black color of the rhabdome in sections stained with iron hæmatoxylin is the presence of these numerous nerve fibrils. Concerning the distribution of these fibrils inside the rhabdome, I am unable to say anything definite, but they probably extend almost straight to the centre. In material fixed in Kleinenberg's picro-sulphuric fixing fluid the rhabdome sometimes appears as a tube, and this may indicate that these fibrils do not run all the way to the centre. While the innervation of the ommatidium is under discussion, it might be stated that there is no indication of nervous connection with any of the other cells peripheral to the retinula.

The significance of the single retinular nucleus which lies at a lower level than the others of each ommatidium, is somewhat hard to explain. Hickson held that some of the retinular cells of *Musca* had more than one nucleus. In this form there are three layers of nuclei in place of two, as in *Apis*. Hesse, on the contrary, homologizes these lower nuclei, only one of which is present in *Apis*, with the proximal retinular cells of the apterygote insect eyes. In these forms the retinula is divided into two parts, one distal to the other, each of which acts alone in the formation of rhabdome structure, and both have nerve fibre connections with the optic ganglia. A similar condition is found in some pterygote insect ommatidia. Of these two views the one of

Hesse seems more probable. As Hickson says, there is nothing morphologically wrong with the supposition that certain cells are multinucleate; but since the explanation of Hesse helps us to complete the homologies of the cells of the ommatidia of the various groups, it seems to have more weight.

The question as to the method of modification in number of retinular cells during the course of evolution is an interesting one, but it must be admitted that as yet very little is known concerning it. It seems not unlikely that the ommatidium of the bee is changing either from eight to nine retinular cells, or from nine to eight, since it is rather rare for the number of these elements to be variable. The thought has suggested itself that possibly this one proximal nucleus was one which was in the process of delamination from the ommatidial epidermis, and was therefore tending toward a reduction of retinal elements, but this does not seem to be as probable an explanation as that of Hesse. It may be said, however, that Johansen describes the ommatidium of *Vanessa* as having seven retinular cells and two retinal ganglion cells, while in *Apis* there is probably but one retinal ganglion cell to each ommatidium and at least one more cell in each retinula.

VII.—HOMOLOGIES OF COMPONENT PARTS.

The question of homologies of the various eyes of the invertebrates has excited much discussion, but since only compound eyes have been investigated in this paper, this problem will not be taken up here. The question of the homology of the different kinds of compound eyes is worthy of consideration. Such eyes occur in crustacea and insects,[4] and a comparison of the groups indicates that there is here either uniformity of origin and plan or one of the most remarkable cases of convergence known in the animal kingdom. The essential part of the ommatidium is the retinula, and this may be considered as a sense bud, formed by the accumulation of cells sensitive to light, which has been modified internally to aid in light perception. Since such groups of cells occur throughout the whole animal kingdom and associated with all the senses, there is nothing remarkable about the similarity so far. In addition to the retinula, an ommatidium consists of a cone and a chitinous covering which may be faceted, and possibly accessory cells occur between ommatidia which act as pigment cells. In order that the light rays may be centred on the retinular nerve fibres, some refractive organ must be present above it (the cone) and the whole organ must be covered by chitin, as is the rest of the body. This chitin in turn may assist in the refraction, as it does in many cases, or may even secondarily assume the functions of the cone entirely if no cone substance is differentiated (acone eyes). For the occurrence of these parts there are but two explanations: either they are differentiations of cells which formerly lay outside the retinula group, and have been placed distal to it to assist in collecting light rays to form a more perfect image, or they have been placed distal to the retinula by the differentiation of some other cell layer which has been superimposed.

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[4] No account of the so-called compound eyes of myriapods and arachnids is taken here, since their plan is so different that they cannot readily be homologized with those of crustaceans or insects. Until we know more of the comparative embryology of these forms it may be as well to suspend judgment. I do not feel qualified to include these in the present discussion, but evidently from the researches of numerous investigators we may conclude that the homology is not as close as in the forms under discussion.

The various cells of the ommatidium seem to lend themselves to homologies very readily. The retinulæ of the various ommatidia are groups of cells which are the nerve endings of the eye, and all ommatidia agree in this respect. Retinulæ of apterygote insects, some crustacea and a few pterygote insects have two layers of retinular cells, while others have but one, but, as was pointed out for *Apis*, the position of nuclei at different levels in the higher insects may indicate a remnant of a former two-layered condition for these retinulæ also. In other words, the morphological invagination by which the insect eye has arisen may be carried farther in some cases than in others. Hickson has shown that acone, pseudocone and eucone cones are probably homologous, and the fact that some cones are composed of but two cells while others have four seems a matter of small moment. The probable homology of the corneal hypodermal cells of apterygote insects and crustacea with the corneal pigment cells of most pterygote insects has been dwelt on sufficiently and is held on comparative anatomical grounds by Hesse. The accessory pigment cells are undoubtedly but undifferentiated cells of the layer of epidermis from which the retinulæ arise, and their presence or absence is of small importance in homologizing the different ommatidia. The fact also that mesodermal cells may migrate to a position between ommatidia, as is held for some eyes, is also of small consequence. As far, then, as the component parts of the ommatidia are concerned there is no difficulty about establishing a very close homology, and this similarity is considerably strengthened by showing that the corneal pigment cells are not only similar in function to the corneal hypodermal cells, but that at an early stage they actually occupy the same position.

The whole question seems, then, to be one which must be settled from embryological evidence. The problem is, which of the two methods of formation previously mentioned is the method which actually exists in ontogony, and are all compound eyes formed by the same method? From this work on *Apis* and that of Johansen on *Vanessa* it is evident that the differentiation of cells outside the retinula to form cone and lens layers is what occurs in insects, and the whole question hinges on the development of the crustacean eye. Reichenbach and Kingsley describe the eye as arising by an invagination; and if either of these investigators is right, although they differ as to the fate of the three layers formed, then the compound eyes of these crustaceans are not homologous with the compound eyes of insects. On the other hand, Herrick insists that the compound eye of *Alpheus* arises from a single layer of epidermis, and according to this view the homology holds. Herrick's view, that even if an invagination does occur it is of no importance, does not seem tenable, for if an invagination occurs then cone and retinula do not come from contiguous cells, and that I believe to be a matter of great importance.

From the striking similarity in position and function of the parts of the ommatidium, and from the observations of Herrick, we are safe in concluding that the eyes of the various groups under consideration are distinctly homologous, and there must be some other explanation for the invaginations observed by the other writers mentioned.

The interpretation of the formation of the ommatidium which is held from an examination of the eye of *Apis* makes possible a very close homology of the elements of the compound eye with the ocelli of insects, such as was held by Grenacher; and this homology seems materially strengthened since an homology can be shown between the corneal pigment cells of insect ommatidia with the chitin-secreting cells of the ocelli. An objection that might be raised is that the vitreous body of the ocellus arises from cells which are all to one side of the retina rather than from all sides, but since they are adjoining cells this might be a secondary change. From sections of ocelli of the pupae of the bee which have been examined, it is evident that the middle ocellus arises from a double invagination, indicating a fusion of two organs, while the lateral ocelli arise from single invaginations.

VIII.—SUMMARY.

The primitive arrangement of ommatidia is tetragonal (p. 130).

The hairs over the lens are secreted by bi-nucleated hair cells with intracellular ducts which lie between the ommatidia (p. 131).

The ommatidium arises as a group of cells with superimposed nuclei which later become arranged as a spindle surrounded by smaller cells (p. 136).

This spindle is the retinula, and the cone cells and pigment cells assume a distal position by a morphological invagination (p. 137).

The retinula is the centre of the ommatidium, and the cone cells, corneal pigment cells and outer pigment cells follow in the order named (p.141).

The ommatidium is composed of eight or nine retinula cells around the rhabdome, four cone cells, two corneal pigment cells and about twelve outer pigment cells (p. 126).

The rhabdome and cone are intracellular secretions, while the lens is an extracellular secretion of the pigment cells (p. 144).

The corneal pigment cells are homologous with the corneal hypodermal cells of crustacean and apterygote insect ommatidia (p. 147).

The innervation of the ommatidium is by a differentiation of part of the retinular cells into nerve fibrils, and these extend to the retinular ganglia (p. 149).

The lens is secreted by the corneal pigment cells which early in the pupa stage lie distal to the cone, and possibly also by the outer pigment cells (p. 148).

Pigment is formed inside all the cells of the ommatidium, except the cone cells, by a cytoplasmic differentiation (p. 149).

The ommatidium arises from a strictly one-layered epidermis, which passes directly from the larva to the pupa without the loss of any cells or additions from other tissues (p. 136).

LITERATURE.

BALFOUR, F. M. 1881. *A Treatise on Comparative Embryology*, II, London.

BÜTSCHLI, O. 1870. Zur Entwicklungsgeschichte der Biene. *Zeit. f. Wiss. Zool. XX*, pp. 519–564.

CARRIÈRE, J. 1884. On the Eyes of Some Invertebrates. *Qt. Jr. Micr. Sc.*, XXIV, New Series, pp. 673–81.

—— 1885. Die Sehorgane der Thiere vergleichend anatomisch dargestellt. München und Leipzig, R. Oldenbourg, 6 + 205 pp.

—— 1885. Einiges über die Sehorgane von Arthropoden. *Biol. Centralbl.*, V, No. 19, pp. 589–97.

—— 1886. Kurze Mittheilungen aus fortgesetzten Untersuchungen über die Sehorgane. *Zool. Anz.*, IX, Nos. 217 and 230.

FERNALD, H. T. 1890. The Relationships of Arthropods. *Studies Biol. Lab. Johns Hopkins Univ.*, IV, pp. 431–513.

GRENACHER, H. 1874. Zur Morphologie und Physiologie des facettirten Arthropodenauges. *Göttingen Nachrichten*, pp. 645–56.

—— 1877. Untersuchungen über das Arthropoden-Auge. *Beilageheft zu den klinischen Monatsblättern für Augenheilkunde*, XV.

—— 1889. Untersuchungen über das Sehorgan der Arthropoden, insbesonder des Spinnen, Insecten und Crustaceen. Göttingen, Vanderhoeck und Ruprecht, 8 + 188 pp., 11 Taf.

Herrick, F. H. 1889. The Development of the Compound Eye of Alpheus. *Zool. Anz.*, XII, pp. 164–9.

Hesse, Rich. 1901. Untersuchungen über die Organe den Lichtempfindung bei niederen Thieren. VII, Von den Arthropoden-Augen. *Zeit. f. wiss. Zool.*, LXX, pp. 348–473.

Hickson, S. J. 1884. The Eye and Optic Tract of Insects. *Qt. Jr. Mic. Soc.*, No. 98.

—— 1885. The Retina of Insects. *Nature*, XXXI, pp. 441–2.

Johansen, H. 1893. Die Entwicklung des Imagoauges von Vanessa urticæ L. *Zool. Jahrbüch*, VI, pp. 445–80.

Kenyon, F. 1896. The Brain of the Bee. *Jr. Comp. Neurology*, VI, pp. 133–210.

—— 1897. The Optic Lobes of the Bee's Brain in the Light of Recent Neurological Methods. *Am. Nat.*, XXXI, pp. 369–76.

Kingsley, J. S. 1886. The Arthropod Eye. *Am. Nat.*, XX, pp. 862–7.

—— 1886. The Development of the Compound Eye of Crangon. *Zool. Anz.*, IX, No. 234, pp. 597–600.

—— 1887. The Development of the Compound Eye of Crangon. *Jr. Morph.*, I, No. 1, pp. 49–66.

Leydig, F. 1855. Zum feineren Bau der Arthropoden. *Arch. f. Anat., Physiol. u. wiss. Med.*, pp. 376–480.

—— 1864. Das Auge der Gliederthiere. Tübingen, Laupp und Siebeck, 50 pp.

Lowne, Thompson B. 1878. On the Modifications of the Simple and Compound Eyes of Insects.

—— 1884. On the Compound Vision and the Morphology of the Eye of Insects. *Trans. Linn. Soc.*, London.

Parker, G. H. 1891. The Compound Eyes in Crustaceans. *Bull. Mus. Comp. Zool. Harvard Univ.*, XXI, No. 2, pp. 45–140, 10 plates.

Patten, William. 1886. Eyes of Molluscs and Arthropods. *Mitth. Zool. Stat. Neapel*, VI, pp. 542–756.

—— 1887. Studies on the Eyes of Arthropods. I. Development of the Eyes of Vespa. *Jr. Morph.*, I, pp. 193–226.

—— 1890. Is the Ommatidium a Hair-bearing Sense-bud? *Anat. Anz.*, V, Nos. 13, 14, pp. 353–9.

Reichenbach, H. 1886. Studien zur Entwickelungsgeschichte des Flusskrebses. Frankfurt.

Watasé, S. 1889. On the Structure and Development of the Eyes of the Limulus. *Johns Hopkins Univ. Circ.*, VIII, No. 79, pp. 34–7.

—— 1890. On the Morphology of the Compound Eyes of Arthropods. *Studies Biol. Lab. Johns Hopkins Univ.*, IV, No. 6, pp. 287–334.

Willem, V. 1897. Les yeux et les organes post-antennaires des Collemboles. *Ann. Soc. entomol. de Belgique*, XLI, p. 225.

Abbreviations.

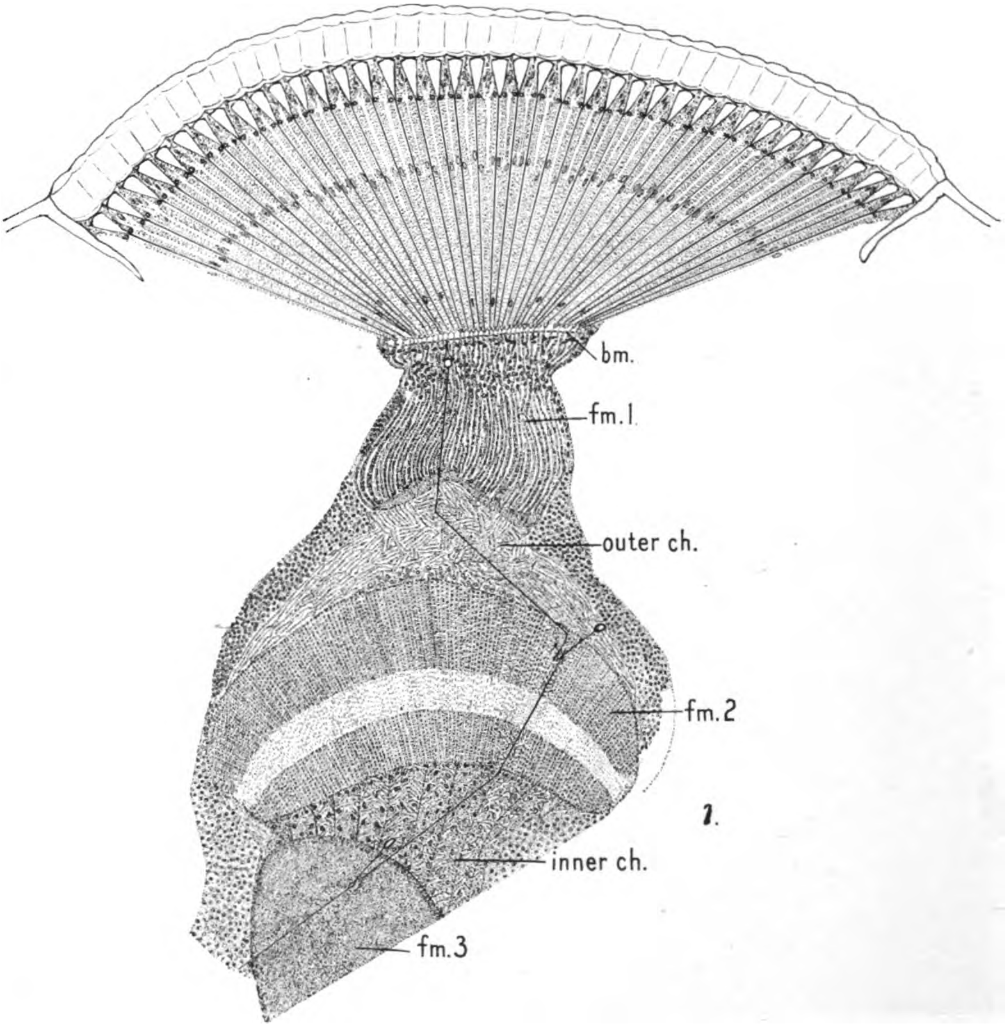
l., lens.
c. p. c., corneal pigment cell.
o. p. c., outer pigment cell.
b. m., basement membrane.
l. ret. n., lower retinular nucleus.
ch., chitin.
f. b., facet boundary.
ret. gang. n., nucleus of retinular ganglion.
br. sh., brain sheath.
int. d., intracellular duct.
c. c., crystalline cone.
rhb., rhabdome.
ret., retinula.
pgm., pigment.
n., nucleus.
h. c., hair cell.
tr., trachea.
n. f., nerve fibre.

EXPLANATION OF PLATES VI, VII, VIII.

PLATE VI, Fig. 1.—Section of entire eye and optic lobes. The heavy lines show the course of the nerve fibres as worked out by Kenyon (diagrammatic).

PLATE VII, Fig. 2.—Ommatidium of young pupa before rhabdome is differentiated and at time of first pigmentation of retinula cells.
Fig. 3.—Ommatidium of older pupa, showing differentiation of rhabdome and lens formation.
Fig. 4.—Cross-section through distal end of cone of pupa of same age as fig. 2, showing corneal pigment cells.
Fig. 5.—Cross-section through cone of older pupa.
Fig. 6.—Cross-section through proximal end of cone of pupa (same stage as figs. 3 and 5).
Fig. 7.—Cross-section through cone of young pupa.
Fig. 8.—Cross-section through retinula of young pupa before rhabdome formation.
Fig. 9.—Cross-section through distal end of retinula of young pupa, showing first traces of pigment.

PLATE VIII, Fig. 10.—Entire ommatidium (somewhat diagrammatic). Adult.
Fig. 11.—Entire ommatidium, as if dissected out, without outer pigment cells (diagrammatic). Adult.
Fig. 12.—Section of entire ommatidium, showing distribution of pigment. Adult.
Fig. 13.—Cross-section just proximal to lens, slightly oblique.
Fig. 14.—Cross-section through extreme distal ends of retinulæ and proximal ends of cones, slightly oblique.
Fig. 15.—Cross-section through retinulæ, showing relation of outer pigment cells in this region.
Fig. 16.—Cross-section through retinulæ in region of nuclei.
Fig. 17.—Cross-section through retinulæ in region of proximal nucleus.
Fig. 18.—Cross-section of eye, cutting basement membrane parallel. The distinctness of nerve fibres of each ommatidium is shown.



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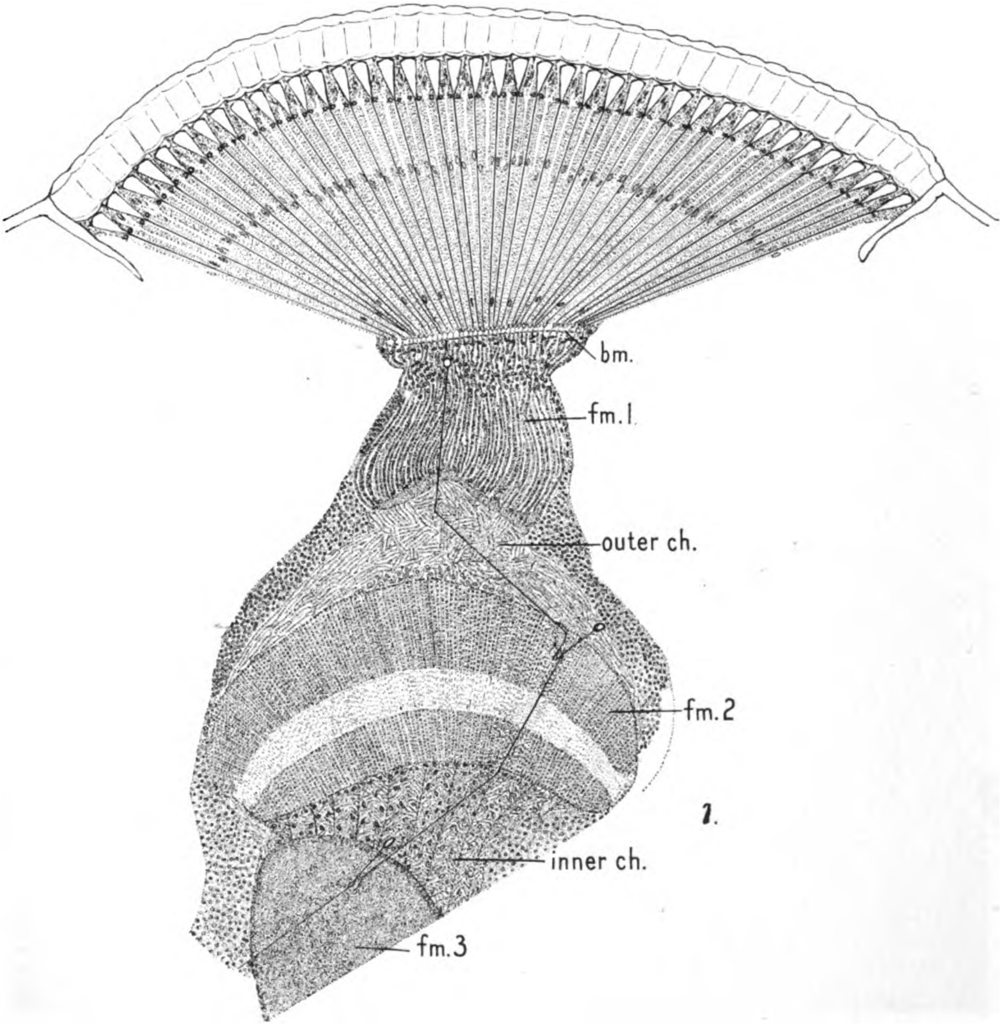

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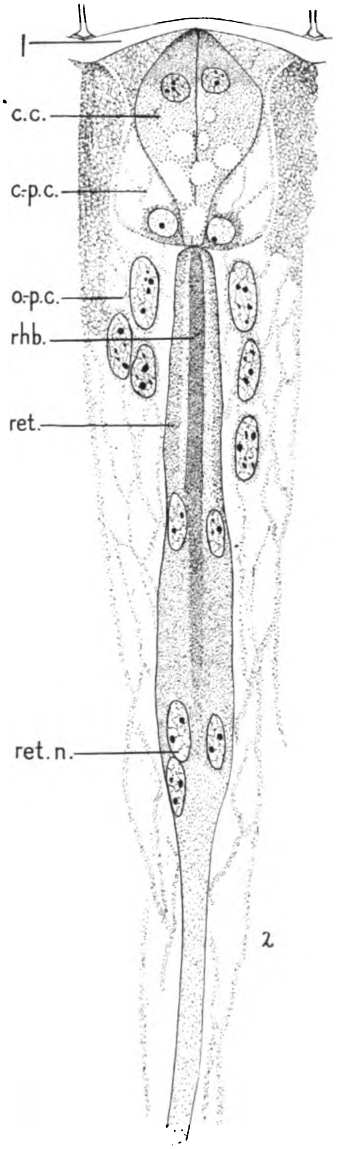

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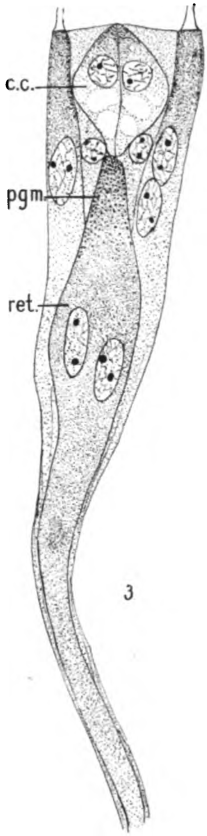

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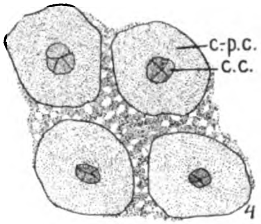

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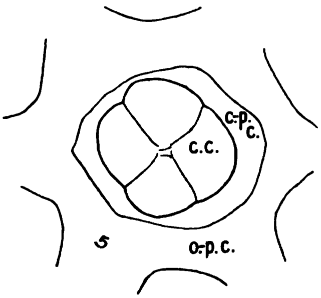

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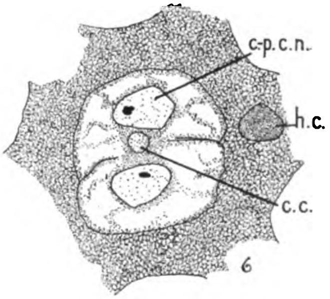

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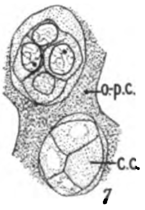

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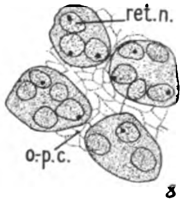

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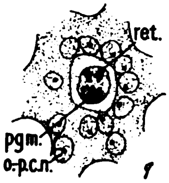

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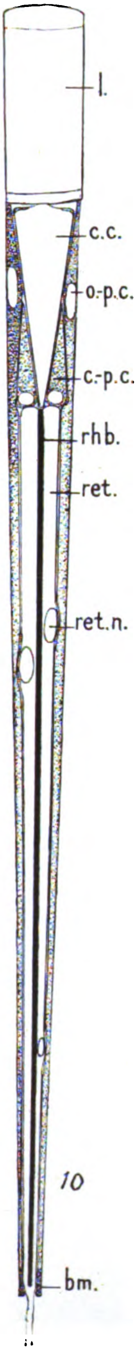

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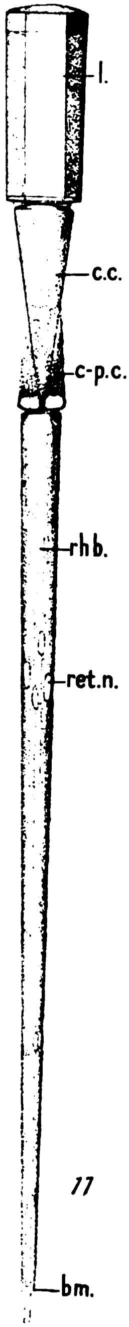

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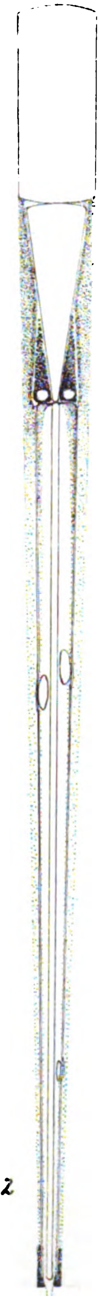

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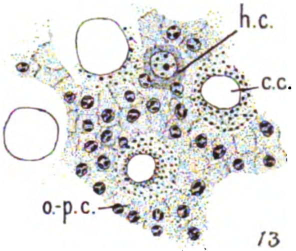

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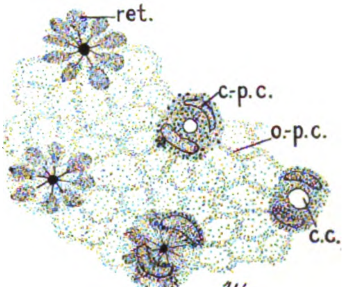

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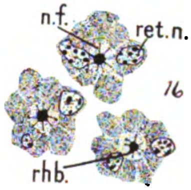

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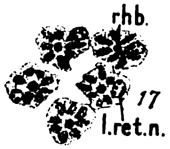

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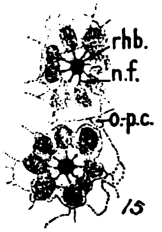

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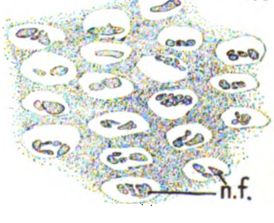

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